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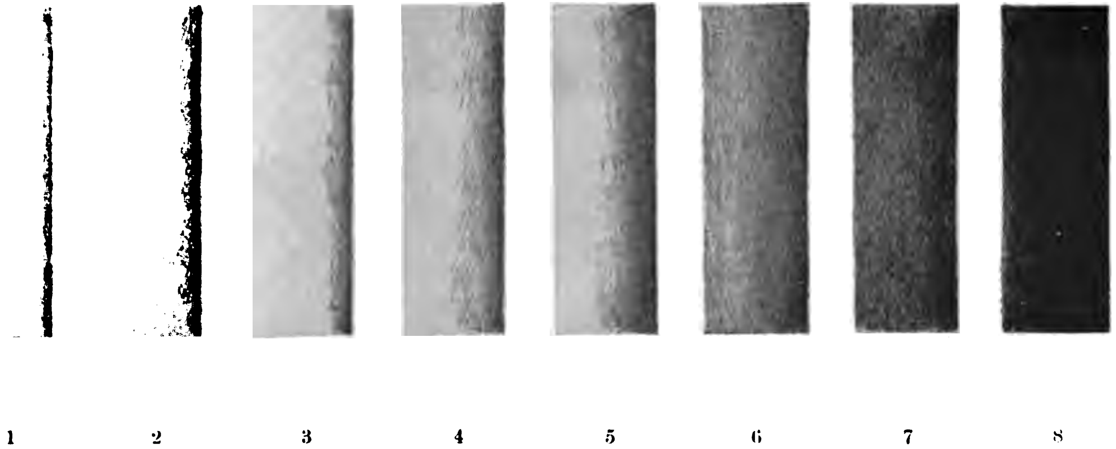

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FLESH FOODS

WITH METHODS FOR

THEIR CHEMICAL, MICROSCOPICAL, AND BACTERIOLOGICAL EXAMINATION

A Practical Hand-Book for
Medical Men, Analysts, Inspectors, and others

BY

C. AINSWORTH MITCHELL, B.A. (Oxon.), F.I.C., F.C.S.
MEMBER OF COUNCIL, SOCIETY OF PUBLIC ANALYSTS

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TO

OTTO HEHNER, Esq.,

PAST-PRESIDENT OF THE SOCIETY OF PUBLIC ANALYSTS,
VICE-PRESIDENT OF THE INSTITUTE OF CHEMISTRY,

As a mark of Regard and Esteem.

PREFACE.

THE word 'flesh,' like so many other words in the English language, has come to have a very extended application, and although in its strictest sense it is applied to the muscular tissue only, yet it is often used to connote the combined muscular and connective tissue (including fat and bone), or even the whole of the interior organs and tissues of an animal. It is with flesh in the sense of muscular fibre that this book chiefly deals, although the connective tissue and blood are touched upon as being intimately associated with the muscle.

It has been the author's endeavour to collect and summarise in a convenient form, records of investigations which are, for the most part, scattered throughout English and foreign scientific books and periodicals, and to select such methods as appeared most suitable for the examination of meat and its preparations.

In describing these methods an elementary knowledge of analytical chemistry and bacteriology on the part of the reader has been assumed, so as to save the space which would have been required for details which may be found in any general text-book.

The author gratefully acknowledges the valuable assistance given him by many friends in the preparation of this book, and especially by Mr. Otto Hehner, and by Dr. Sykes, editor of the *Analyst*, to whom he is also indebted for the loan of numerous preparations of the parasites of flesh.

He would also express his best thanks to Mrs. E. Mitchell and Mr. R. M. Prideaux, F.I.C., for their kindness in drawing many of the illustrations; to Dr. König for permission to make use of the numerous tables published in his classical work, *Chemie der menschlichen Nahrungs- und Genussmittel*; and to Dr. H. Schjerning for the communication of the results of unpublished work.

C. A. M.

57 Chancery Lane, London, W.C.,
May 1900.

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FLESH FOODS.

CHAPTER I.

STRUCTURE AND CHEMICAL COMPOSITION OF MUSCULAR TISSUE.

STRUCTURE OF MUSCLE.

CLASSIFICATION OF MUSCULAR FIBRES.

Flesh, in its primary sense of *muscular contractile tissue*, consists of numbers of fibres lying side by side, and united by means of connective tissue carrying the nerves and blood-vessels. These muscular fibres may be classified into three groups in accordance with their appearance under the microscope.

1. Unstriated involuntary muscle of the alimentary canal, blood-vessels, intestines, bladder, etc.
2. Striated involuntary muscles of the heart.
3. Striated voluntary muscles.

1. Non-Striated Muscle.—The unstriated or smooth muscular fibres, which are not under the control of the will, are composed of a number of small fibre cells, which are usually spindle-shaped and contain an elongated nucleus, the ends of which are surrounded by granular protoplasm. Although usually described as unstriped muscle, a longitudinal striation may frequently be observed in the fibres, especially after they have been treated with reagents.

2. Cardiac Muscle.—This occupies an intermediate position, as regards structure, between the non-striated and striated muscle. It consists of elongated and branched cells, each of which contains a nucleus, and is marked by faint longitudinal and rough transverse striations.

3. Voluntary Striated Muscle.—The transversely striped muscular fibres, which, from the fact that they compose the muscular tissue under the control of the will, are often described as voluntary muscles, consist of long contractile fibres about $\frac{1}{500}$-inch in diameter, and as much as an inch or more in length.

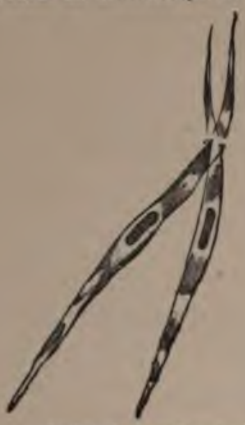

Fig. 1.—Isolated smooth muscle. × 300. (*Stirling.*)

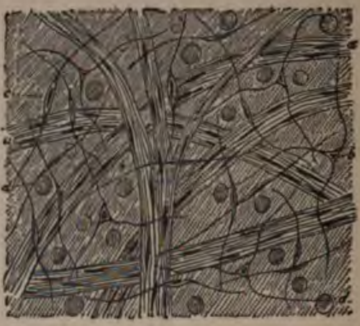

Fig. 2.—Strands of smooth muscle from Bladder of Frog.

Each fibre is surrounded by an elastic envelope or *sarcolemma*, which is a structureless proteid body. On breaking a fibre in two, the ends of the sarcolemma may often be left attached to the two fragments. The fibres, with their surrounding sarcolemma, are bound together by a variety of connective tissue, in which lies a deposit of fat. From the interior of the fibre a viscous liquid

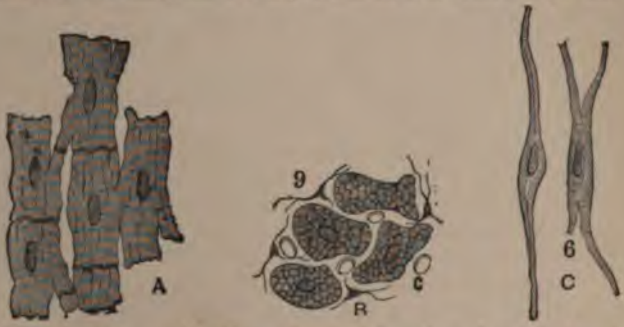

Fig. 3.—Cardiac muscular fibre. A, muscular fibres from the heart of a mammal, and C, from a frog; B, transverse section of the cardiac fibres; *b*, connective tissue corpuscles; *c*, capillaries. (*Landois and Stirling.*)

which readily congeals to a soft jelly can be expressed. This is known as *muscle plasma.*

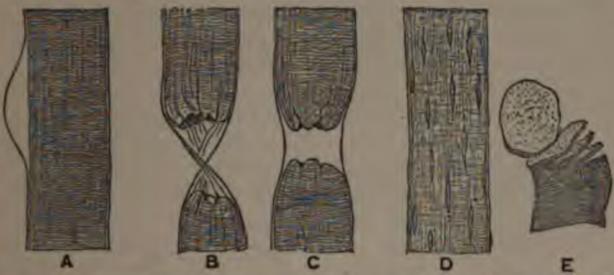

FIG. 4.—Striped muscle of Frog. A, sarcolemma raised; B and C, ruptured fibres; D, fibre treated with acetic acid; E, muscle discs. (*Stirling.*)

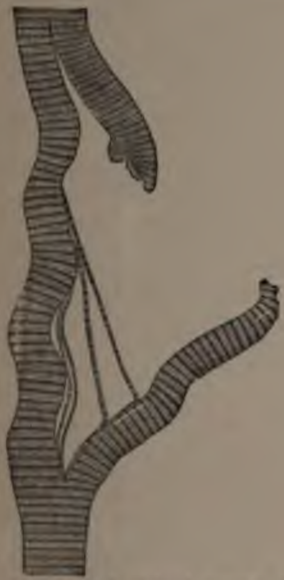

FIG. 5.—Part of striped muscle of Calf, teased, showing isolated bundles of fibrils. × 200. (*Stirling.*)

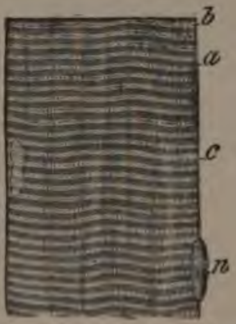

FIG. 6.—Muscular fibre of great adductor of Rabbit, living and extended. *a*, dim disc; *b*, light disc; *c*, intermediate or Dobie's line; *n*, nucleus seen in profile. Examined in its own juice. × 300. (*Stirling.*)

Voluntary muscle shows but little vertical striation, but has characteristic transverse markings composed of alternating dark and bright lines. When examined under higher powers of the microscope, the lighter stripe shows a further division, a thin black line, known as *Krause's membrane*, making its appearance. In the darker stripes a region less dark than the rest may also be observed. This is known as *Hensen's disc.*

Striated muscle, after being steeped in alcohol or chromic acid, can be resolved into *fibrillæ*, in each of which alternately light and dark transverse markings are visible. Horizontal cleavage can be brought about by macerating the fibres in dilute hydrochloric acid, which creates a tendency to split across through the bright bands, into a number of discs termed *discs of Bowman.* The numberless small particles which would be produced by a simultaneous vertical and horizontal cleavage were called by Bowman * *sarcous elements*—a term which is now used in a different sense.

Sub-division of Voluntary Muscle.—The voluntary muscles of some animals show distinct differences in appearance, some being *pale* and others *red.* In the pale muscles, an example of which is seen in the breast muscles of a hen, the striations are well marked, and the longitudinal markings very faint; in the red muscles the vertical striations are much more plainly visible. The red muscles contract more slowly than the pale muscles, the fibres are thinner, and they contain more sarcoplasm.

Double Refraction of Muscle.—The property of double refraction is a characteristic of muscular fibre, and is readily demonstrated by means of the polariscope. It is especially marked in the case of striated fibre, though also clearly discernible in the smooth variety.

Muscle Plasma.—When dead muscular fibre is examined, all the contents of the sarcolemma are solid; but by rapidly freezing living muscular fibre and applying pressure, it is possible to express a viscous liquid which readily congeals to a soft jelly. This is known as *muscle plasma*, and it is the coagulation of this plasma which causes the rigidity of muscular tissue, or *rigor mortis*, which rapidly sets in after death. The coagulation is retarded by cold, but only in the case of cold-blooded animals is it possible to thus delay it sufficiently to enable the plasma to be collected. According to Kühne the contents of the sarcolemma consist of sarcous elements suspended in this liquid, and the changes which these bodies undergo in their form cause the contraction of the muscle.

---

* *Phil. Trans. Roy. Soc.*, 1840, p. 457.

CHEMICAL COMPOSITION OF MUSCLE: I. ORGANIC COMPOUNDS.

A.—PROTEID CONSTITUENTS.

The Sarcolemma.—This is composed of a proteid substance which is somewhat related to that of elastic tissue, from which, however, it differs in being slowly dissolved by acids and alkalies, and in being more readily acted upon by peptic and pancreatic enzymes.

Muscle Plasma.—*Preparation.*—Kühne's method of preparing this is to free the muscular tissue of a frog from blood by injection of a solution of salt (0·5 per cent.) into the aorta, and to treat the fibre cut into small fragments, at 0° C., with more of the salt solution to eliminate the lymph. The fragments are then frozen by exposure to a temperature of $-7°$ C., cut into slices with chilled knives, pounded in a cold mortar, and pressed in linen at the ordinary temperature. The expressed liquid, which has a temperature of about 0° C., is filtered through paper moistened with ice-cold salt solution.

Proteids of Muscle Plasma.—Neumeister * gives a description of these, of which the following is a summary:—

Myogen fibrin.—On warming muscle-plasma to about 40° C. coagulation takes place—a proteid substance, named *myogen fibrin* by Fürth,† being deposited. The amount of this varies in different animals, a larger quantity being obtained from frogs' muscle, for instance, than from that of mammals.

Myosin.—On dialysing the plasma, from which the myogen fibrin has been removed, for twelve to twenty-four hours, in running water, and subsequently in distilled water, a voluminous precipitate is obtained. This proteid, termed *myosin* by Fürth, can also be precipitated by adding ammonium sulphate until the solution contains 23 per cent. It is of a globulin character.

Myosin fibrin.—When a neutral aqueous solution of myosin, containing salt, is allowed to stand, it gradually becomes turbid and deposits a flocculent precipitate—*myosin fibrin.* This is insoluble in neutral liquids, and is regarded as an insoluble modification of myosin. It is completely precipitated from neutral solutions of myosin at 50° C.

Myogen.—Myosin composes only about 20 per cent. of the proteids of plasma (rabbits'), the remaining 80 per cent. consisting of a proteid not precipitated by dialysis—*myogen.* It can be isolated by complete saturation of the muscle plasma with ammonium sulphate after the myosin has been removed by partial saturation. It is not a globulin. On adding acetic acid or mineral acids to solutions of myogen, and then neutralizing, *syntonin* is precipitated.

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* *Phys. Chem.*, p. 401.

† *Arch. Exper. Path. u. Pharm.*, 1895, 36, p. 231, etc.

Soluble myogen fibrin appears to be an intermediate stage in the formation of myogen fibrin.

Neumeister gives the following genetic scheme of the production of muscle fibrins from the plasma, and considers that some such change probably takes place in the alteration which occurs in muscle after death:—

Myosin.	Myogen.
|	|
Myosin fibrin.	Soluble myogen fibrin.
	|
	Myogen fibrin.

Myoproteid.—Fürth has isolated from the muscle-plasma of fish a proteid substance which he terms *myoproteid.*

Nuclëins.—These are not present in great quantity in muscular fibre. In dogs' muscle they amount to about 0·37 per cent. The muscles of embryos, the composition of which is more akin to that of young cells, contain more.

They are compound albuminous substances in which phosphoric acid is a principal constituent, in combination, in certain cases, with bases such as guanine, xanthine, etc. Neumeister gives the elementary composition of nuclëin obtained from the yolk of egg as:—Carbon, 42·11; hydrogen, 6·08; nitrogen, 14·73; oxygen, 31·05; sulphur, 0·55; phosphorus, 5·19; and iron, 0·29 per cent.

Nuclëins are usually insoluble in water and alcohol, but dissolve in dilute alkalies. On treatment with boiling acids or alkalies they yield derivatives of the proteid part of the molecule together with phosphoric acid, and, in certain cases, nuclëin bases and their derivatives.

Phospho-Carnic Acid.—Siegfried precipitated with ferric chloride from muscle extract previously freed from albumin, a compound containing phosphorus, to which he gave the name of phospho-carnic acid, and which he considered was expended during muscular activity. It can be salted out with ammonium sulphate, and, on dialysis into water, decomposes, yielding phosphoric acid.

Enzymes of Muscle.—The enzymes pepsin, ptyalin, and maltase have been identified in muscle. It is also probable that other enzymes are present, and the coagulation of the proteids of the plasma, and the acidity of the muscle after death, may possibly be brought about through the agency of bodies of this nature.

Colouring Matter of Muscle.—*Hæmoglobin.*—The principal colouring matter of the red muscles is hæmoglobin, which Kühne * was the first to practically demonstrate as being present even in muscular tissue washed completely free from blood. As was mentioned before, † there is a difference in colour in the muscles in different parts of the same animal, and Ranvier ‡ and others have shown that the red colour is a product of the activity of the muscles. Thus, muscles which are continually contracting, like those of the heart, are of a deep red colour, whilst those which are in a comparative state of rest are paler. In birds, for instance, the breast muscles used in flying are dark red, except when, as in the case of the common hen, these are but rarely exercised. Even in the muscles of the same animal, an increase of colour often accompanies an increase of strength—witness the difference in the colour of the flesh of the calf and cow. According to Ranvier the muscle hæmoglobin is not derived from the muscle substance, but originates in the blood-vessels.

Colouring Matter of Fish Muscle.—In the muscular tissue of many kinds of fish (*e.g.* salmon and goldfish), there is, in addition to hæmoglobin, a peculiar rosy red colouring matter, which Krukenberg and Wagner § have found, in the case of the salmon, to be of the nature of a red lipochrome, and not a proteid substance.

Myohæmatin.—This is one of a number of pigments (*histohæmatins*) discovered by MacMunn || in the muscles of many kinds of animals, and notably in the cardiac muscles of the pigeon. Though giving characteristic spectra they have never been isolated. Myohæmatin appears to be capable of forming compounds analogous to the oxyhæmoglobin and methæmoglobin derived from hæmoglobin. MacMunn's theory is that these pigments retain the oxygen which the blood brings to the tissue, until it is required by the latter.

Stroma Substance.—This is a simple proteid substance, which cannot be extracted from the sarcoplasmic bodies by neutral reagents. On treatment with dilute potassium hydroxide solution it passes into solution as an albuminate.

B.—NITROGENOUS NON-PROTEID CONSTITUENTS.

Meat Extractives.—When muscular fibre is extracted with boiling water, a considerable proportion of the proteid substances coagulate, and there pass into solution various non-proteid nitrogenous substances, together with other organic bodies and inorganic salts. The nitrogenous bases, to which Gautier gave the name of

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* *Virch. Arch.*, 1865, 33, p. 79.

† P. 4.

‡ *Arch. Phys.*, 1874.

§ *Zeit. Biol.*, 1885, 8, 37-40.

|| *Phil. Trans. Roy. Soc.*, 1886, Pt. I.

leucomaines or physiological alkaloids, are formed normally in the living cell at the expense of the lecithins or of other nitrogenous compounds (see page 217).

Classification of Leucomaines.—Gautier classifies the leucomaines into five groups:—

I. NEURINIC LEUCOMAINES, including choline, neurine, and betaine.
II. KREATINIC BASES, including kreatine, kreatinine, crusokreatinine, etc.
III. XANTHIC BASES, including adenine, xanthine, sarcine, etc.
IV. LEUCOMAINES OF THE FATTY ACID SERIES, *e.g.* neuridine, cadaverine, gerontine.
V. AMIDO-ACIDS.

I. **Neurinic Leucomaines.**—As a rule these bases are only found in small quantities in animals. They also occur as ptomaines in the products of the putrefaction of animal matter (*cf.* page 218). In both cases they appear to be derivatives of lecithins.

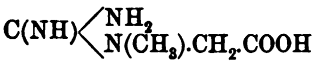
Choline [$C_5H_{15}NO_2$] has been found in small quantity in blood, in glands, and in the yolk of egg.

Neurine [$C_5H_{13}NO$] generally accompanies choline in traces.

Betaine [$C_5H_{13}NO_3$] occurs as a normal constituent in certain molluscs, such as the mussel.

II. **Kreatinic Bases.**—The general characteristics of these bases are that they are usually only slightly soluble in water, and nearly insoluble in alcohol. All are precipitated on adding zinc chloride to solutions of their hydrochlorides. All give precipitates with silver nitrate, and most of them with mercuric chloride. They are distinguished from the xanthic bases by containing more hydrogen, and by not being precipitated by copper acetate.

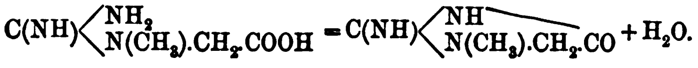
Kreatine [$C_4H_9N_3O_2$] (or Methyl-glycocyamine)—



$$\mathrm{C(NH)}\left\langle\begin{array}{l}\mathrm{NH_2}\\\mathrm{N(CH_3).CH_2.COOH}\end{array}\right.$$

is a feeble base discovered by Chevreul in 1835 in meat broth. It crystallizes in colourless needles and in rhombs which melt at 100° C. It is very soluble in boiling water, less soluble in alcohol, and insoluble in ether.

When an acidified solution of kreatine is boiled it is completely converted into its anhydride, kreatinine.



$$\mathrm{C(NH)}\left\langle\begin{array}{l}\mathrm{NH_2}\\\mathrm{N(CH_3).CH_2.COOH}\end{array}\right. = \mathrm{C(NH)}\left\langle\begin{array}{l}\mathrm{NH}\\\mathrm{N(CH_3).CH_2.CO}\end{array}\right. + \mathrm{H_2O}.$$

When boiled with barium hydroxide it is hydrated, and yields urea and sarcosine.

$$C_4H_9N_3O_2 + H_2O = CO(NH_2)_2 + (NH).CH_3.CH_2.COOH.$$

Gautier considers that this reaction explains the disappearance of the flesh bases from the tissues.

Kreatine is precipitated by sodium phosphomolybdate, and by zinc chloride in the presence of hydrochloric acid and alcohol. Its hydrochloride [$C_4H_9N_3O_2.HCl$] crystallizes in prisms which are non-deliquescent, and but little soluble in alcohol.

It gives no precipitate with Bouchardat's reagent (I + KI), and no blue coloration with a mixture of ferric chloride and potassium ferricyanide.

Method of Separation.—Kreatine can be isolated from an aqueous extract of meat by boiling, filtering, adding a slight excess of basic lead acetate, or of barium hydroxide, filtering, removing the excess of lead by hydrogen sulphide, or of barium by carbon dioxide, filtering, concentrating the liquid at a low temperature, and purifying, by recrystallization, the fine needles which gradually deposit.

Kreatine has a slightly bitter taste. It is not very poisonous when injected into animals, but can be transformed by bacteria into the poisonous ptomaine, methyl-guanidine (page 307). It is a common constituent of the muscles of most animals, the average amount found by C. Voit* being 0·21 to 0·28 per cent. He obtained the following quantities from the muscular fibre of various animals:—Frog, 0·21 to 0·35; fox, 0·206 to 0·237; ox, 0·219 to 0·276; dog, 0·223 to 0·248; rabbit, 0·269 to 0·336; and man, 0·282 to 0·301 per cent. There appears to be less kreatine present in cardiac muscles than in the voluntary muscles.

Kreatinine [$C_4H_7N_3O$].—This base is invariably present in small quantities in the muscles of the higher animals. In certain fish (*e.g.* conger) Krukenberg found as much as 0·3 per cent. In certain diseases, such as pneumonia and typhoid fever, the amount obtainable from the urine is largely increased.†

Kreatinine forms brilliant prismatic crystals which are readily soluble in cold water (12 parts) and in alcohol (120 parts). In aqueous solution it gradually undergoes hydration, being converted into kreatine; and the same result is obtained by treating it with dilute alkalies.

It is precipitated by sodium phosphomolybdate from acid solutions, and by picric acid when the solution is not too dilute. Zinc chloride precipitates it in crystalline needles $[(C_4H_7N_3O)_2ZnCl_2]$, which are nearly insoluble in cold water, and insoluble in alcohol.

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* *Zeit. Biol.*, 1868, iv. p. 77.

† G. S. Johnson has shown that the kreatinine obtained from urine is not identical with the flesh-kreatinine. *Proc. Roy. Soc.*, xlii. p. 365.

It gives a precipitate with mercuric chloride, but not with Bouchardat's reagent $(I + KI)$, or with Selmi's reagent

$$(Fe_2Cl_6 + K_3Fe(CN)_6).$$

It is converted by oxidizing agents into methyl-guanidine, and when heated with barium hydroxide in excess yields methylhydantoin.

Weyl's Reaction for Kreatinine.—On adding to a cold solution of kreatinine several drops of a dilute solution of sodium nitroprusside, and then a little dilute sodium hydroxide solution, a red coloration is obtained which changes to yellow, and on acidifying the liquid with acetic acid and warming becomes green and then blue. This reaction is also given by substances allied to kreatinine which contain the group $[CH_2.CO]$ united to two atoms of nitrogen.

Kreatinine has a greater physiological effect than kreatine.

Iso-Kreatinine.—J. E. Thesen[*] has isolated this base from the muscle of the haddock. It differs from kreatinine in its colour (yellow crystals), in its solubility in various solvents, reducing action on cupric compounds, and in yielding ammonia instead of methylguanidine on oxidation with potassium permanganate. It appears to be converted into kreatinine when allowed to stand in contact with milk of lime.

Xantho-Kreatinine $[C_5H_{10}N_4O]$.—This base was discovered by Gautier[†] in 1882 in muscle and in meat extract. It has often been mistaken for kreatinine, which it resembles in many respects.

It crystallizes in yellow spangles, and has a slightly bitter taste, and an odour recalling acetamide. It dissolves in hot concentrated alcohol, and is fairly soluble in cold water. On heating it gives off ammonia and methylamine. Its reaction is amphoteric.

Its hydrochloride crystallizes in feathery masses. The platinochloride is very soluble. Zinc chloride gives a yellowish-white precipitate, consisting of groups of needles. Silver nitrate gives a gelatinous precipitate, and mercuric chloride a yellowish-white precipitate.

No precipitates are given by potassium mercury iodide, cupric acetate, or iodine in potassium iodide.

Xantho-kreatinine is poisonous when injected in fairly large quantity, causing extreme lassitude, defecation, and vomiting.

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[*] *Zeit. phys. Chem.*, 1897, 24, pp. 1-17.

[†] *Les Toxines*, 1896, p. 236.

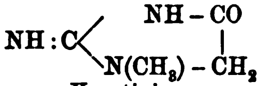
Cruso-Kreatinine [$C_5H_{18}N_4O$].—Gautier discovered this base in muscle in association with xantho-kreatinine.

It crystallizes in orange-coloured lamellæ, which are slightly alkaline and rather bitter.

The hydrochloride is soluble and non-deliquescent. The aurochloride is crystalline and granular, and only dissolves with difficulty. The base is precipitated by ordinary alum, but not by zinc acetate. It is also precipitated by zinc chloride and mercuric chloride, and in acid solution gives a yellow precipitate with sodium phosphomolybdate.

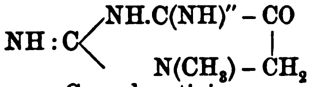
It is not precipitated by potassium mercury iodide, by cupric acetate, or by iodine in potassium iodide, nor does it give Selmi's Prussian-blue reaction.

Gautier gives the following formula representing its constitution compared with that of kreatinine:—



$$NH:C\begin{cases} NH-CO \\ N(CH_3)-CH_2 \end{cases}$$

Kreatinine.



$$NH:C\begin{cases} NH.C(NH)''-CO \\ N(CH_3)-CH_2 \end{cases}$$

Cruso-kreatinine.

Amphi-Kreatinine [$C_9H_{19}N_7O_4$].—This is less soluble than cruso-kreatinine or xantho-kreatinine, which it usually accompanies.

It crystallizes from boiling water in oblique, yellow prisms. It has a slightly bitter taste and weak basic properties. When heated to 100° C. it decrepitates and becomes white and opaque. The hydrochloride is crystalline and non-deliquescent. The aurochloride is trimorphic and very soluble.

The base is not precipitated by copper acetate or mercuric chloride.

It resembles kreatinine in its physiological characters.

Bases [$C_{11}H_{24}N_{10}O_5$] and [$C_{12}H_{25}N_{11}O_5$].—The first of these was found by Gautier in the mother-liquid from which the xantho-kreatinine had been crystallized, and the second in the mother-liquid of cruso-kreatinine.

They resemble kreatinine in their properties.

III. **Xanthic Bases.**—These bases have both basic and acid properties. They contain the group [C : NH], and when heated with alkalies most of their nitrogen is converted into hydrocyanic acid. As a rule they are not directly hydrated by dilute acids or alkalies with the formation of urea. They are very stable, and can, in many cases, be transformed into one another.

Tests for Xanthic Bases.—Most of the xanthic bases, when evaporated with strong nitric acid, leave a residue, which, on treatment with an alkali, turns orange, red, rose-colour, or brownish-yellow. This distinguishes them from the kreatinic bases.

When mixed with chlorine water containing a trace of nitric acid and evaporated to dryness, a residue is obtained, which becomes orange-red or blood-red on the addition of ammonium hydroxide, and with sodium hydroxide often changes to blue.

These leucomaines are also known as 'nuclëin bases,' from the fact that some of them have been found in the nuclëins.

Adenine [$C_5H_5N_5$].—This base was discovered in 1885 by Kossel, who extracted it from vegetable tissues and from the glandular tissues of young animals (αδήν = gland), where it was invariably accompanied by guanine. Although not found in the aqueous extract of muscle, it may be suitably described here.

*Method of Extraction.**—The finely divided pancreas is extracted with water acidulated with sulphuric acid, the sulphuric acid removed from the extract by precipitation with barium, and the filtrate concentrated to about a tenth of its volume *in vacuo* at 50° to 60° C. The liquid is rendered alkaline with ammonia, treated with ammoniacal silver nitrate, and the resulting precipitate washed by decantation and drained on a porous tile. It is then dissolved in ammonium hydroxide (sp. gr. 1·1) containing a little urea. On filtering and cooling, the adenine silver-salt crystallizes out, together with some guanine and hypoxanthine. The precipitate is washed, decomposed, under pressure, with hydrogen sulphide, the liquid filtered and concentrated, and the residue treated with ammonia in not too great excess. As the ammonia evaporates, adenine and guanine are precipitated, while sarcine remains in solution. The precipitate is taken up with warm hydrochloric acid, and on standing, guanine hydrochloride crystallizes out first. On concentrating the filtrate, the adenine hydrochloride is gradually deposited.

Adenine can also be separated from sarcine by converting both into nitrates and concentrating the solutions, when sarcine is deposited as the free base while adenine nitrate (which is not decomposed by evaporation) remains in solution.

Adenine crystallizes in transparent hexagons, which are soluble in 1086 parts of water, and more soluble in alcohol and glacial acetic acid. When heated with potassium hydroxide, it gives hydrocyanic acid. When evaporated with nitric acid it does not give an orange coloration on adding sodium hydroxide to the residue. The hydrochloride is crystalline and dissolves in 42 parts of water. The mercuro-chloride [$(C_5H_5N_5)_2.HgCl_2$] is a fine grey powder which is insoluble in hot dilute hydrochloric acid. The nitrate $[(C_5H_5N_5.HNO_3)_2 + H_2O]$ crystallizes in needles. The picrate can be crystallized from boiling water. The platino-chloride $[(C_5H_5N_5HCl)_2.PtCl_4]$ crystallizes in small yellow needles.

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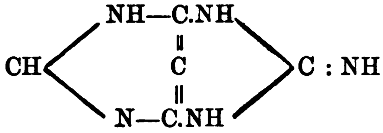
* Gautier, *Les Toxines*, p. 250.

Adenine gives no precipitate with potassium ferro- or ferricyanide. With copper sulphate it yields an amorphous grey precipitate, which dissolves in dilute acids and alkalies, and with ferric chloride produces a red coloration, which does not disappear on heating the solution. When adenine sulphate is heated on the water bath with sodium nitrite it is transformed into sarcine.

Gautier gives the following constitutional formula for this base:—



According to Guareschi it passes through the body into the urine unchanged.

Adeno-Sarcine $[C_5H_5N_5.C_5H_4N_4O]$.—This compound is obtained as a starch-like mass when hot solutions of adenine and sarcine are mixed in molecular proportions and allowed to cool, and can be crystallized in needles from an ammoniacal solution. The compound hydrochloride is more soluble than the hydrochloride of either of the constituents.

Guanine $[C_5H_5N_5O]$.—This base was discovered by Unger in 1844 in guano, whence it derives its name. It usually occurs only in very small quantity in muscular tissue and in various glands. Kossel extracted 0·005 per cent. from beef and still less from dogs' flesh. In the muscle of embryos, however, and in the flesh of certain lower animals, such as the cuttlefish, it has been found in larger proportion.

It is a white amorphous powder, sparingly soluble in water, and readily soluble in acids, but insoluble in alcohol and ammonium hydroxide.

The hydrochloride $[C_5H_5N_5O.HCl + H_2O]$ crystallizes in fine needles, which lose their water of crystallization at 100° C. and their hydrochloric acid at 200° C. The platino-chloride forms orange crystals, which are sparingly soluble in water. The sulphate forms long yellow needles, which are decomposed by water. Mercuric chloride forms an insoluble precipitate with it $[(C_5H_5N_5O.HCl)_2.HgCl_2 + H_2O]$, and potassium ferrocyanide precipitates it in crystalline needles.

Nitrous acid converts it into xanthine

$$C_5H_5N_5O + HNO_2 = N_2 + H_2O + C_5H_4N_4O_2.$$

When guanine is evaporated with nitric acid, then mixed with a little potassium hydroxide and evaporated to dryness, it gives the indigo coloration which xanthine gives under the same conditions.

Guanine is not poisonous. In its passage through the system it is partly decomposed, with the formation of urea and uric acid.

Pseudo-Xanthine [$C_4H_5N_5O$].—Gautier discovered this base in muscle in association with kreatinine, sarcine, xantho-kreatinine, and cruso-kreatinine.

Method of Separation.—It is isolated from meat extract by precipitating part of the bases with 95 per cent. alcohol, evaporating the alcoholic filtrate, and taking up the residue with strong alcohol. From this solution various leucomaines are precipitated by ether. The mother-liquid of these, when boiled with copper acetate, gives a precipitate, which is decomposed with hydrogen sulphide. The liquid is filtered boiling, and the crystals, which are deposited on cooling, are dissolved in hydrochloric acid,* a crystalline hydrochloride of the same form as that of sarcine being produced.

The free base resembles xanthine in its physical and chemical properties.

It is soluble in alkaline liquids. On evaporating it with nitric acid, and taking up the residue in water, an orange coloration is obtained.

Silver nitrate precipitates it as a gelatinous salt, but no precipitate is obtained with lead acetate. Its mercuro-chloride is very soluble in hydrochloric acid.

Sarcine or Hypoxanthine [$C_5H_4N_4O$].—This was first discovered by Scherer in the spleen, and afterwards by Strecker in muscle. The amount usually present in voluntary muscle varies from 0·07 to 0·12 per cent. It accompanies adenine and guanine in the tissues of many glands, and has been found in the blood of certain fish. Gautier suggests that in the organism it may be derived in part from guanine, which gives, on oxidation, sarcine, xanthine, and uric acid.

Method of Separation.—It can be isolated from meat extract in the following manner:—After the nitrates of adenine and hypoxanthine have been obtained (*cf.* page 12) their solution is nearly neutralized, and a slight excess of picric acid added. Adenine picrate separates as a flocculent yellow precipitate, while hypoxanthine picrate remains in solution.

On adding silver nitrate to the boiling filtrate, the compound $C_5H_3AgN_4O.C_6H_2(NO_2)_3$ is obtained. This is decomposed with hydrochloric acid, and the picric acid extracted with ether. The hydrochloride of hypoxanthine is left in solution, and on the addition of sodium carbonate, the base is precipitated.

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* *Les Toxines*, p. 260.

Sarcine is a white crystalline powder, more soluble than xanthine in boiling water, in which it forms a neutral solution. It dissolves in alkalies, and sublimes at 150° C. without decomposing.

Its hydrochloride ($C_5H_4N_4O.HCl + H_2O$) crystallizes in bright prisms. Its mercuro-chloride forms a flocculent precipitate which dissolves in acids.

It is precipitated from acid solutions as a phosphomolybdate, but is not precipitated by picrates.

When oxidized with nitric acid it does not yield xanthine (Gautier), though the latter base is produced with permanganate as the oxidizing agent. Pure sarcine does not give the murexide test. It has a marked physiological action. When 50 to 100 milligrammes were injected into a frog, tetanic convulsions were produced after six to twenty hours. In a chicken it increased the secretion of uric acid (Gautier).

Xanthine [$C_5H_4N_4O_2$].—This base, which almost always accompanies sarcine and adenine, especially in glandular tissue, was discovered by Marcet in a urinary calculus in 1823. The quantity present in muscular fibre varies considerably. In the flesh of pigeons and hens, Kossel found from 0·01 to 0·1 per cent.

Method of Separation.—Gautier gives the following method of separating it from an extract of the flesh. The extract is dissolved in as little hot water as possible, and precipitated with an excess of 95 per cent. alcohol. The residue is dissolved in water and treated with lead acetate (not in excess). The liquid is filtered hot, freed from lead, and concentrated by evaporation. Kreatinine crystallizes out first and is filtered off. On adding ammoniacal lead subacetate (not in excess) to the mother liquid, xanthine is precipitated, while hypoxanthine remains in solution. The lead compound is decomposed with hydrogen sulphide, and the boiling liquid filtered. Mercuric acetate is added to the filtrate and the liquid boiled. The precipitate is decomposed with hydrogen sulphide, and the liquid again filtered while hot. On evaporating the filtrate, the xanthine separates out as a yellowish crust.

It can be obtained synthetically by heating hydrocyanic acid with water and a slight excess of acetic acid at 145° C.

$$11HCN + 4H_2O = C_5H_4N_4O + C_6H_6N_4O_2 + 3NH_3.$$

Xanthine is soluble in 14,000 parts of cold and 1160 parts of boiling water. It is insoluble in alcohol and ether. It is decomposed at 156° C. with the formation of ammonium cyanide, carbon dioxide, formic acid, and glycocoll. Under the influence of nascent hydrogen it is converted into sarcine.

Its hydrochloride [$C_5H_4N_4O_2.HCl$] crystallizes in needles and hexagonal plates. The platino-chloride forms soluble yellow prisms.

The Murexide Test.—On treating xanthine with a little nitric acid and evaporating the liquid to dryness, a yellow residue is obtained, which, on the addition of potassium hydroxide, becomes red, and changes to violet-blue on heating.

When xanthine is treated with a mixture of a solution of an alkaline hypochlorite and sodium hydroxide, an olive to dark green colour is produced, which subsequently changes to brown and disappears. This test distinguishes xanthine from sarcine.

Physiologically xanthine resembles sarcine. When injected into a frog it causes muscular contractions and paralysis of the spinal chord.

Heteroxanthine [$C_6H_6N_4O_2$] (or Methyl-xanthine).—This base is found in small quantity in dogs' urine, and possibly occurs in the flesh. It can be separated from paraxanthine by means of ammonia water, in which it is readily soluble.

Paraxanthine [$C_7H_8N_4O_2$] (or Di-methyl-xanthine) was found by Salmon in 1883 in normal human urine. It is isomeric with theobromine.

Carnine [$C_7H_8N_4O_3$].—This base was discovered in 1871 by Weidel in American meat extract, but was not found in the muscular tissue itself. It has since been found in normal urine by Pouchet, and in the muscular tissue of fish by Krukenberg and Wagner.

Method of Separation.—It can be isolated by the following method:—The extract of meat is dissolved in water, treated with barium hydroxide (not in excess), and filtered. The filtrate is precipitated with lead subacetate, and the precipitate taken up with boiling water, which dissolves the combination of carnine and lead oxide. The lead is removed from the filtrate, the liquid filtered boiling, and silver nitrate added to it after concentration and cooling. The silver carnine compound is digested with an excess of ammonia, which dissolves the silver chloride simultaneously precipitated. The silver is removed from the residue with hydrogen sulphide, the filtrate evaporated and decolorized with animal charcoal, and the carnine obtained by crystallization.

Carnine is a white crystalline base with a neutral reaction and bitter taste. It is hardly soluble in cold water, and is insoluble in alcohol and ether, but is easily soluble in hot water. On treatment with bromine water or nitric acid it is converted into hypoxanthine.

$$C_7H_8N_4O_3 + 2Br = C_5H_4N_4O.HBr + CH_3Br + CO_2.$$

It is distinguished from xanthine, hypoxanthine, and guanine in yielding with basic lead acetate a precipitate which dissolves completely in boiling water. With copper acetate it gives a bluish-green precipitate, and with mercuric chloride a white one. It is not precipitated by picric acid.

Carnine hydrochloride crystallizes in needles, while the platino-chloride forms a yellow powder.

According to Neumeister it has no characteristic colour reactions when quite free from xanthine.

Physiologically it has not a pronounced injurious effect on the system. It appears to resemble caffeine as a muscular stimulant, and to affect the heart if taken in too great excess.

IV. **Leucomaines of the Fatty Acid Series.**—These are also found among the products elaborated by various bacteria.

Trimethylamine.—This occurs normally in many tissues and secretions (*cf.* p. 218). It appears to be derived from the lecithins.

Neuridine.—This base has been found in the human brain and in the yolk of egg, together with neurine and choline (*cf.* p. 305).

Cadaverine.—Has been found in fresh pancreas.

Gerontine [$C_5H_{14}N_2$].—This base, which is isomeric with the two preceding, was extracted by Grandis from the liver of a dog.

It crystallizes in needles which are soluble in water and in alcohol.

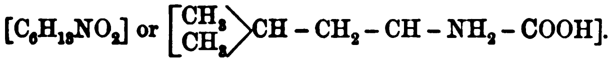
Its hydrochloride forms small deliquescent crystals, which are soluble in alcohol. The platino-chloride crystallizes in large needles, very soluble in water.

Physiologically it has a paralyzing effect on the nerve centres, but does not affect the muscles.

V. **Amido Acids.**—**Glycocoll** or **Amido-acetic Acid** [$CH_2.NH_2 - COOH$].—This occurs in large quantity in the edible mussel and in the flesh of several mammalia. It is one of the decomposition products of collagene (pp. 23 and 154). It dissolves readily in water, but is insoluble in alcohol and ether. By adding freshly precipitated copper hydroxide to a hot concentrated solution of glycocoll, blue crystals of copper glycocoll are deposited on cooling.

Sarcosine or **Methyl-glycocoll** [$CH_2(NH - CH_3) - COOH$], although it does not appear to occur in the animal system, may be mentioned here as a derivative of kreatinine, which is converted by hydration into sarcosine and urea.

Leucine or **Amido-caproic Acid**



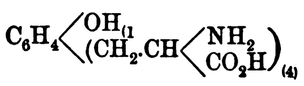
$$[C_6H_{13}NO_2] \text{ or } \left[\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>CH - CH_2 - CH - NH_2 - COOH\right].$$

This is a proteid derivative, which occurs in the thyroid and other glands, in the pancreas, and in the blood of certain animals. It crystallizes in brilliant plates which dissolve readily in water, and are fairly soluble in hot alcohol. It is optically active, rotating the beam of polarized light to the right, but by the action of certain mould-fungi it is converted into a lævo-rotatory modification.

The leucines formed in the pancreatic digestion of various proteids were found by R. Cohn * to consist of several individuals.

Butalanine or **Amido-valeric Acid** is another proteid derivative found among the products of pancreatic digestion and in the pancreas itself.

Tyrosine [$C_9H_{11}NO_3$] or **Para-hydoxyphenyl-α-amido-propionic Acid**



$$C_6H_4\left\langle\begin{array}{l}OH_{(1}\\(CH_2.CH\left\langle\begin{array}{l}NH_2\\CO_2H\end{array}\right.)_{(4)}\end{array}\right.$$

This substance accompanies leucine and other amide bodies in the liver, pancreas, etc., and is formed in the decomposition of proteid substances, especially in the digestion. It crystallizes in glistening needles, melting at 295° C. It is fairly soluble in hot water, but insoluble in alcohol and ether.

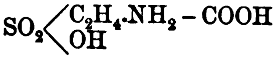
It has feeble basic and acid properties.

Its hydrochloride is crystalline, very acid, and is dissociated by water.

The platino-chloride is deliquescent.

Tyrosine gives a red coloration or precipitate when boiled with Millon's reagent (p. 161).

Taurine or **Amido-ethyl-sulphonic Acid**



$$SO_2\left\langle\begin{array}{l}C_2H_4.NH_2-COOH\\OH\end{array}\right.$$

is an amido-acid with feeble basic properties. It has been found in minute quantities in the muscles of various kinds of animals, as for instance in those of the horse and of molluscs. It crystallizes in large brilliant prisms which are slightly soluble in cold water, but insoluble in alcohol and ether.

Lecithins.—There appear to be several kinds of lecithins yielding different proportions of fatty acids on decomposition. In 1846 a complex substance was extracted by Gobley from the yoke of egg, and similar substances have since been found in the brain, in fish-roe, in blood, and in many animal tissues.

Method of Separation.—Gautier † gives the following method of isolating lecithin. The yolk of the egg is extracted with ether, the extract evaporated, the residue taken up with petroleum spirit, and the liquid filtered. The filtrate is shaken several times with

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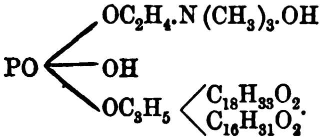
* *Zeit. phys. Chem.*, 1895, **20**, p. 203.

† *Les Toxines*, p. 276.

75 per cent. alcohol, the petroleum spirit expelled, and the solution left for several days. Cholesterin and a little lecithin separate. The clear liquid is decanted, decolorized with charcoal, and evaporated *in vacuo* at 50° C. to a syrup. The residue is taken up with ether, which on evaporation leaves nearly pure lecithin, and the latter is purified by crystallization from as small a quantity as possible of absolute alcohol at 5° C. It rapidly undergoes alteration, especially on heating. When treated with alkalies or acids it is decomposed with the formation of glycero-phosphoric acid, stearic and oleic acids, choline and other bases.

According to Strecker, its formula is $C_{42}H_{84}NPO_9$ or



$$PO \begin{cases} OC_2H_4.N(CH_3)_3.OH \\ OH \\ OC_3H_5 \begin{cases} C_{18}H_{33}O_2 \\ C_{16}H_{31}O_2 \end{cases} \end{cases}$$

Inosinic Acid.—According to Haiser,* the formula of this substance is $C_{10}H_{18}N_4PO_8$. The free acid is decomposed by boiling water into hypoxanthine, trioxyvaleric acid, and phosphoric acid. It is an amorphous substance, though it forms crystalline salts with alkalies. It was first found by Liebig in beef, and has since been found in varying quantity in the muscle of rabbits, cats, birds, and fish. The greatest amount recorded (0·21 per cent.) was found by Creite in the muscle of the turkey.†

C.—NON-NITROGENOUS ORGANIC CONSTITUENTS.

Reaction of Muscle.—The reaction of living muscle is neutral or slightly alkaline. But after death, and soon after *rigor mortis* has set in, it becomes decidedly acid, until, with the commencement of decomposition, it again becomes gradually alkaline from the formation of ammonia and substituted ammonias. By plunging the living muscle into boiling water or alcohol the production of free acid is to a large extent prevented. A high temperature accelerates the process, whilst cold retards or prevents it. The presence of oxygen appears to be non-essential, for the acidity occurs as rapidly *in vacuo*, or when the muscle is kept under oil or mercury, as in the open air. It is possibly due to the action of an enzyme. On the reaction of the muscular tissue Eber has based a test of the fitness of flesh for human food.‡

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* *Ann. Chem. Pharm.*, 158, 1871, p. 353.

† Neumeister, *loc. cit.*, p. 439.

‡ See p. 75.

Free Acids of Dead Muscle.—The chief acid causing the acid reaction is a characteristic ethylidene lactic acid

$$(CH_3 - CH.OH - COOH),$$

which differs from the isomeric fermentation product in being optically active (+). Another lactic acid is also present, which is probably identical with the fermentation acid. The total quantity of lactic acid varies from 0·1 to 1 per cent.

This lactic acid was formerly regarded as being derived from glycogen, but Neumeister * cites various authorities against this view. Salkowski † holds that the lactic acid is a waste product of the activity of the living muscle, being carried away by the blood, and that death puts a stop to the process of its formation. He regards its production as a function of living protoplasm, and not as the action of an enzyme.

Glycogen ($C_6H_{10}O_5$).—This is a reserve material invariably present in muscular fibre. By the activity of the protoplasm, or of an enzyme, it is constantly being converted into glucose, which, by decomposition and oxidation, serves as a source of strength.‡ This conversion does not immediately cease on the death of the muscle. According to Nasse's experiments § the quantity of glycogen in the resting muscles of a frog is on the average 0·43 per cent. In rabbits it amounts to from 0·47 to 0·95 per cent. (*cf.* p. 134).

Fat.—Neumeister || regards this as a further reserve material. It is present in the connective tissue, between the fibres and in the sarcoplasma.

Glucose.—This is produced by the action of the protoplasm on the glycogen, which passes through the successive stages of erythrodextrin, achroodextrin, maltose, and glucose. If muscular tissue be instantly placed in hot water after being taken from an animal the activity of the protoplasm (or enzyme) is arrested, and only a trace of glucose will be detected.

Inosite ($C_6H_{12}O_6 + 2H_2O$) is a non-fermentable isomer of glucose first discovered by Scherer in cardiac muscular tissue.¶ Small quantities are usually present in both striated and smooth muscles. It is optically inactive, and does not reduce metallic oxides or Fehling's solution, though it changes the colour of the latter to green. According to Jacobsen, horse flesh contains about 0·003 per cent. of inosite.

Scherer's Test for Inosite.—On adding strong nitric acid to a

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* *Loc. cit.*, pp. 412-16.

† *Zeit. f. klin. Med.*, 1890, **17**, Suppl. 21.

‡ Neumeister, *loc. cit.*, p. 424.

§ *Pflüg. Arch.*, pp. 97-121.

|| *Loc. cit.*, p. 425.

¶ *Ann. Chim. Pharm.*, 1850, p. 322.

solution of pure inosite, evaporating to dryness on the water bath, moistening the residue with ammonia and calcium chloride solution, and again evaporating to dryness, a rose-red coloration is obtained.

Scyllite.—This substance, which is closely allied to inosite in chemical composition, is found in the muscles of certain fish.

CHEMICAL COMPOSITION OF MUSCLE: II. INORGANIC CONSTITUENTS.

Mineral Matter.—The muscular tissue of the higher animals contains from 1 to 1·5 per cent. of mineral matter, chiefly potassium phosphate. The ash left on incineration also contains sodium, magnesium, iron, calcium, chlorine, and traces of sulphates, probably derived from the decomposition of proteid matter on ignition.

The subjoined table of analyses by Katz * gives the proportion of mineral constituents in the flesh of different animals:—

MINERAL CONSTITUENTS OF FLESH.

Flesh of	Water.	Potassium Oxide.	Sodium Oxide.	Ferric Oxide.	Calcium Oxide.	Magnesium Oxide.	Phosphoric Acid (P_2O_5).				Chlorine.	Sulphur.
							Total.	Soluble in Water.	Soluble in Alcohol.	Residue.		
Pig, . . .	72·89	0·306	0·210	0·008	0·011	0·046	0·487	0·350	0·085	0·053	0·048	0·204
Ox, . . .	75·80	0·441	0·088	0·035	0·003	0·040	0·389	0·279	0·065	0·046	0·057	0·187
Calf, . . .	75·39	0·458	0·166	0·013	0·020	0·050	0·503	0·334	0·097	0·072	0·067	0·226
Deer, . .	75·27	0·405	0·095	0·015	0·013	0·048	0·569	0·411	0·096	0·062	0·040	0·211
Rabbit, .	76·83	0·479	0·067	0·008	0·026	0·048	0·579	0·469	0·068	0·043	0·051	0·199
Dog, . . .	76·42	0·392	0·127	0·006	0·010	0·039	0·512	0·345	0·110	0·055	0·081	0·227
Cat, . . .	75·14	0·456	0·097	0·013	0·012	0·047	0·461	0·351	0·066	0·043	0·057	0·219
Hen, . .	68·38	0·560	0·128	0·013	0·015	0·061	0·580	0·456	0·057	0·067	0·060	0·292
Frog, . .	81·62	0·371	0·074	0·009	0·027	0·039	0·426	0·349	0·047	0·030	0·040	0·163
Shellfish, .	80·60	0·403	0·133	0·008	0·031	0·044	0·313	0·263	0·029	0·021	0·241	0·223
Eel, . . .	63·10	0·290	0·043	0·008	0·055	0·030	0·405	0·336	0·046	0·023	0·034	0·135
Pike, . .	79·83	0·301	0·040	0·006	0·056	0·051	0·485	0·392	0·036	0·058	0·032	0·248

Water.—The muscles of young animals always contain more than those of older animals, whilst those of embryos contain most of all. In the earliest stages it amounts to not less than 99·4 per cent., which gradually falls to 81·2 per cent.†

The flesh of birds contains somewhat less water, and that of cold-blooded animals rather more than the flesh of mammalia. Neumeister gives the following figures :—

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* *Arch. ges. Physiol.*, 1896, 3, p. 14.

† Neumeister, *loc. cit.*, p. 441.

	Mammalia.	Birds.	Cold-blooded Animals.
Water,	76·5	73·0	78·8
Solid Matter,	23·5	27·0	21·2

Of muscles in different parts of the body the cardiac muscles appear to contain the most water.

Gases in Muscle.—The chief of these is carbon dioxide, the amount of which appears to be quite independent of the presence of oxygen, as much being given off *in vacuo* as in the air. Traces of nitrogen are also present.

SUMMARY OF THE COMPOSITION OF MUSCULAR FIBRE.

The following table is based upon those given by König,[*] Hofmann,[†] and Neumeister.[‡]

		Per Cent.	
	Mammalia.	Birds.	Cold-Blooded Animals.
Water,	74·7–78·3	71·7–77·3	80·0
Solid matter,	21·7–25·5	22·7–28·3	20·0
Organic matter,	20·8–24·5	21·7–26·3	18·0–19·0
Inorganic matter,	0·9– 1·0	1·0– 2·0	1·0– 2·0

Alkaline albuminate,	2·85–3·01		Mammalia.
Proteids insoluble in		Guanine,	0·005
neutral liquids,	14·5 –16·7	Taurine (horse),	0·07
Collagene,	2·0 – 5·0	Inosinic acid,	
Fat,	0·5 – 3·7	Phosphoric acid,	0·34 –0·50
Glycogen,	0·3 – 1·0	Chlorine,	0·067
Lactic acid,	0·04– 1·0	Potassium oxide,	0·40 –0·50
Inosite,	0·003	Sodium oxide,	0·02 –0·08
Kreatine,	0·2 – 0·28	Calcium oxide,	0·008–0·018
Xanthine,	0·01– 0·10	Magnesium oxide,	0·02 –0·15
Hypoxanthine,	0·04– 0·12	Ferric oxide,	0·003–0·01

[*] *Nahr. u. Genussmitt.*, ii. p. 93.

[†] *Lehrbuch der Zoochemie*, p. 104.

[‡] *Phys. Chem.*, p. 443.

CHAPTER II.

STRUCTURE AND COMPOSITION OF CONNECTIVE TISSUE AND BLOOD.

CONNECTIVE TISSUE.

UNDER this name histologists classify a number of substances, which at first sight appear to have but little resemblance to one another. These are:—

- Connective tissue proper, sub-divided into white tissue, and yellow or elastic tissue.
- Adipose tissue.
- Cartilage.
- Osseous tissue, or bone.

CONNECTIVE TISSUE PROPER.

In one or other of its forms connective tissue is present in almost all parts of the body, its function being to connect or hold together adjacent organs or parts of organs. In certain parts it is soft and tender; in others, such as the tendons and ligaments, it is hard, tough, and of great strength. It is composed of numerous fine fibres, some of which lie parallel, while others cross one another. The fibres composing the yellow or elastic tissue are fewer in number than the white fibres, from which they also differ in colour and in their behaviour on treatment with acetic acid, and on boiling with water. The cells of connective tissue primarily contain granular protoplasm, in which a nucleus may be observed.

White Fibres.—These consist of an albuminoid substance, *collagene* (κολλα, glue), which when boiled with water yields gelatin. The latter substance is obtained in quantity by boiling tendons (which are chiefly composed of white fibres) with water, preferably under pressure (*cf.* p. 154).

Elastic Fibres.—These, unlike the white fibres, do not swell up and become transparent on treating the connective tissue with acetic acid, and hence their presence in tissue can be readily demonstrated by means of that reagent. After prolonged boiling with water, the white fibres can be completely dissolved out from the connective tissue in the form of glue or gelatin, leaving behind the elastic fibres, which are composed of an albuminoid substance named *elastin* (*cf.* p. 154).

Mucin.—All connective tissue proper contains a small proportion of this proteid substance, which can be isolated by macerating the tissue for several days in lime water, and precipitating the proteid by the addition of a dilute acid from the alkaline solution thus obtained.

It occurs in larger proportion in embryonic connective tissue, and is a constituent of the mucus with which epithelial cells are covered. It is not digested by an acid solution of pepsin, but dissolves in alkaline trypsin.

According to Fischer and Krause * the specific gravity of connective tissue varies from 1·1189 to 1·1065, the mean being 1·1141.

ADIPOSE TISSUE.

Distribution of Fat in the Body.—Fat is found in the animal body either in a state of solution or suspension in the various fluids, or deposited in cells. In the latter case the cells are often those of the connective tissue, in which the protoplasmic contents have been gradually displaced by the fat, leaving the original cell-walls in their former state. The condition of the fat cells under-

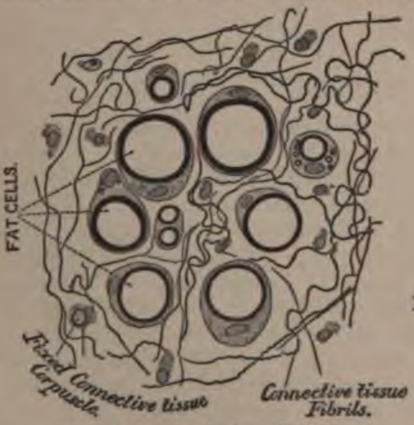
FAT CELLS.
Fixed Connective tissue Corpuscle.
Connective tissue Fibrils.

FIG. 7.—Fat cells from Rabbit. (*Landois and Stirling.*)

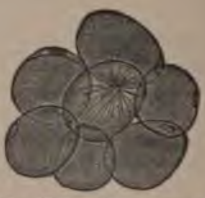

FIG. 8.—Fat cells, some showing nucleus. The central one shows 'margarin' crystals. × 100. (*Stirling.*)

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* Falck, *Das Fleisch*, p. 352.

goes fluctuations according to the state of nutrition and habits of the animal. For instance, it is well known that wild animals possess, as a rule, much less fat than do animals of the same species in captivity. The fat cells usually occur in groups, supported by the fibrous substance of the connective tissue.

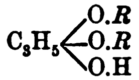
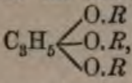
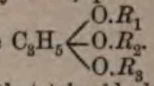
Fischer and Krause give the following figures for the specific gravity of adipose tissue:—Maximum, 0·9254; mean, 0·9232; minimum, 0·9243.

Composition of Animal Fat.—The subject of fats and oils, even when confined to those found in the animal kingdom, is far too wide to be treated of at length in the present work, and for a fuller description the reader must consult one of the numerous manuals dealing specially with their composition and analysis.

Animal fats (excluding milk fat and waxes, such as beeswax and sperm oil) consist almost entirely of compounds of glycerin with higher fatty acids (chiefly palmitic and stearic) of the acetic series ($C_nH_{2n}O_2$), and of compounds with unsaturated acids of different series (chiefly oleic acid), together with traces of lower volatile acids. Judging by the large proportion of iodine absorbed by the more liquid portion of the fatty acids of many animal fats, glycerides of acids more unsaturated than oleic acid must be frequent constituents. Kurbatoff[*] claims to have isolated linolic acid from the fat of the seal and Caspian hare, and Farnsteiner has found it in lard, ox tallow, and horse fat (*cf.* page 101).

Recently Amthor and Zink[†] have found that the fats of certain wild animals and birds, noticeably those of the wild boar, hare, wild rabbit, and blackcock, have remarkable drying properties and very high iodine values, from which facts the presence of a still more unsaturated acid, possibly in the same series ($C_nH_{2n-6}O_2$) as the linolenic acid of linseed oil, may be inferred. This has received confirmation from the work of Farnsteiner (page 101), who has found traces of linolenic acid in lard and tallow.

Amthor and Zink also obtained with several of the lesser known animal fats in a fresh condition (*e.g.* fox, cat, etc.) a high acetyl value, and it is thus not improbable that hydroxylated fatty acids, like the ricinoleic acid of castor oil, may occasionally be normal constituents.[‡]

So far as is known the glycerin in animal fats is always in combination with the fatty acids in the form of triglycerides, and although diglycerides of the type  $C_3H_5\begin{cases} O.R \\ O.R \\ O.H \end{cases}$ are said to have been found in old vegetable oils, they do not appear to have been normal constituents. It is not known with certainty whether the three acid radicles in a triglyceride invariably belong to the same fatty acid, so that the glycerides are of the type  $C_3H_5\left\langle\begin{matrix}O.R\\O.R\\O.R\end{matrix}\right.$, in which R represents the radicle of any one acid, say stearic acid, or whether, in some cases, two of the radicles belong to one acid and the third to another; or whether all three radicles belong to different acids, say oleic, palmitic, and stearic acids, forming glycerides of the type  $C_3H_5\left\langle\begin{matrix}O.R_1\\O.R_2\\O.R_3\end{matrix}\right.$. There is evidence,* however, which tends to prove that tri-acid glycerides exist in butter fat, so that it is not improbable that similar compounds may occur in body fats. As confirmatory evidence it may be mentioned that Hehner and Mitchell have separated bromine compounds from linseed oil and marine animal oils, which have the characteristics of bromides of mixed glycerides.

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[*] *Berichte*, 25, p. 506.

[†] *Zeit. anal. Chem.*, 37, pp. 1–17. *Analyst*, 1897, 22, p. 75.

[‡] *Cf.* note, p. 58.

As to the relative proportion of solid and liquid fatty acids, considerable variations are found not only in the fat of animals of different species, but also in the fat of different individuals of the same species, and even in the fat from different parts of the body of the same animal. In like manner, the amount of stearic and palmitic acid in the solid portion of the fat, and of the oleic and linolic acid in the liquid portion shows enormous variations.† Stearic acid, for instance, may vary from 0 to about 25 per cent. in the fat from different parts of the same sheep,‡ and it is a well-recognized fact that the fat of American pigs contains considerably more of a liquid fatty acid with a greater degree of unsaturation than oleic acid than does the fat of European pigs.§

The composition of fish oils (cod-liver, etc.) and of marine animal oils (whale, etc.) is still more complex than that of land animals, and much of the work that has been done on the subject is either unconfirmed or has been disproved. Speaking generally, fish oils appear, for the most part, to consist of glycerides of various unsaturated fatty acids, together with smaller quantities of the glycerides of saturated acids (palmitic, etc.), some of which are deposited when the oil is cooled below the ordinary temperature. Many of the unsaturated acids are apparently of a different character to those found in the fat of land animals. Cod-liver oil, for instance, appears to contain a fatty acid of the series $C_nH_{2n-6}O_2$, which is not identical with linolenic acid, for Hehner and Mitchell have prepared from that oil an insoluble hexabromide with the same composition as that of the analogous compound to be obtained from linseed oil, but with very different physical properties.*

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* *Analyst*, 1898, p. 317. † *Cf.* p. 54. ‡ *Analyst*, 1896, **21**, p. 327.

§ von Raumer, *Zeit. angew. Chem.*, 1897, pp. 210–215 and 247–254. Twitchell, *Analyst*, 1895, xx. p. 165.

Fahrion,† too, claims to have discovered an unsaturated fatty acid (jecoric acid) with the formula $C_{18}H_{30}O_2$, and another unsaturated acid with the composition $C_{17}H_{32}O_2$ (asellic acid) in certain fish oils.

THE PRINCIPAL ACIDS AND GLYCERIDES OF ANIMAL BODY FAT.‡

Saturated Fatty Acids.	Formula.	Specific Gravity.	Melting Point.	Solidifying Point.	Mol. Weight.	Found in
ACETIC SERIES ($C_nH_{2n}O_2$).						
Palmitic Acid, . .	$C_{16}H_{32}O_2$	0·8527 at $\frac{62°}{4°}$ C.	62° C.	..	256	Most animal fats.
Tri-Palmitin, . .	$C_3H_5(O.C_{16}H_{31}O)_3$	..	62°–64° C.	45°-47° C.	804·2	
Stearic Acid, . .	$C_{18}H_{36}O_2$	0·8454 at 72° C.	71·5° C.	..	284	Most animal fats.
Tristearin, . .	$C_3H_5(O.C_{18}H_{35}O)_3$	..	71·6° & 55°	..	888	
Unsaturated Fatty Acids.						
i. ACRYLIC SERIES, $C_nH_{2n-2}O_2$.						
Oleic Acid, . . .	$C_{18}H_{34}O_2$	0·898 at 14° C.	14° C.	4° C.	282	Most animal fats.
Triolein, . .	$C_3H_5(O.C_{18}H_{33}O)_3$	0·900 at 15° C.	..	..	882	
ii. LINOLIC SERIES, $C_nH_{2n-4}O_2$.						
Linolic Acid, . .	$C_{18}H_{32}O_2$	0·920 C. at 14° C.	Liquid at -18° C.	..	280	Horse fat, lard, ox-tallow.
Trilinolein, . .	..	..	..	..	..	
iii. LINOLENIC SERIES, $C_nH\ _{n-}O_2$.						
(Linolenic Acid), . .	$C_{18}H_{30}O_2$	0·9228 at 15·5° C.§	..	..	278	Lard, ox-tallow, marine animal oils.
(Jecoric Acid), . .	..	..	..	..	..	
iv. HYDROXYLATED SERIES, Series $C_nH_{2n-2}O_3$.						
(*Ricinoleic Acid*), . .	$C_{18}H_{34}O_3$	0·9400 at 15° C.	..	..	..	..

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* *Analyst*, 1898, p. 317. † *Jour. Soc. Chem. Ind.*, 1893, pp. 935 and 938.

‡ This table does not include the fatty acids present in the milk fat of different animals (butyric acid, etc.), since the subject of milk does not come within the scope of the present book.

§ Hehner and Mitchell, *Analyst*, 1898, p. 313.

Many of the fish oils contain considerable proportions of unsaponifiable matter, noticeably *cholesterin*, an alcohol which also occurs, though to a lesser extent, in the fat of land animals.

Sperm oil and bottle-nose oil differ from marine oils properly so called in containing hardly any glycerides, and are, in fact, liquid waxes.

As an examination of the characteristics of the fat attached to or contained in the muscular tissue may often be of use in identifying the kind of flesh, the formulæ and some of the physical constants of the chief fatty acids and triglycerides which have been found or which are likely to be met with in the body fat of some of the terrestrial animals more commonly used as food are given in the accompanying table (see p. 27).

The physical and chemical constants of the fat of various individual animals are given on pages 47–66, and methods for determining these constants in Chapter V.

CARTILAGE.

Structure.—Cartilaginous tissue consists essentially of a groundwork or *matrix* surrounding certain cells which contain protoplasm in which one or two nuclei can be observed. From the different characteristics assumed by the matrix, cartilage has been classified into the following groups:—

Hyaline, in which the matrix is a semi-transparent, homogeneous substance, often resembling ground glass, and occasionally of a fibrinous nature.

Cellular, in which the matrix is transparent, and the envelope or *capsule* surrounding the cells is thin.

White-fibre Cartilage, in which the matrix is composed of a large number of fibres which are apparently of the same nature as those of connective tissue proper.

Elastic or spongy, in which the matrix is made up of a network of elastic fibres.

Composition.—The cartilage taken from different parts of the body varies considerably in composition, as is shown by the results of the analysis made by Hoppe-Seyler.*

	Water.	Organic Matter.	Mineral Matter.
Costal Cartilage, . . .	67·67	30·13	2·20
Articular „ from knee-joint,	73·59	24·87	1·54

The mineral matter of cartilage consists principally of sodium and potassium sulphates, with smaller quantities of sodium chloride and of the phosphates of sodium, calcium, and magnesium.

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* Quoted by Gamgee, *Phys. Chem.*, p. 268.

Chondrin.—The matrix of cartilaginous tissue, when subjected to long-continued treatment with boiling water, yields a glue-like substance formerly known as *chondrin,* but which has been shown to be a mixture of gelatin and soluble alkali salts of *chondroitin sulphuric acid.* According to Mörner,* this proteid, which belongs to the class of glycoproteids, is present in all the varieties of cartilage.

Soluble Proteids.—Besides soluble chondroitin compounds, there are various soluble proteid substances (*e.g.* globulins) always present in cartilage.

Elastin.—This albuminoid is contained in the residue left when part of the cartilage is converted into the so-called *chondrin* by long-continued boiling with water.

'*Albumoid.*'—This is the name given by Mörner † to a characteristic proteid which appears to be an invariable constituent of all adult mammalian cartilage. It is quite insoluble in neutral liquids, and is closely allied to the albuminoids *elastin and keratin,* from which, however, it differs in composition and in being soluble in gastric juice.

OSSEOUS TISSUE, OR BONE.

Structure.—On the exterior of all bones is a fibrous membrane, the *periosteum,* and between the joints of connected bones a layer of cartilaginous tissue. Inside the bone there is often a channel, the *medullary cavity,* the ends of which become broken up by partitions of the bony substance into a large number of very small cavities, the *cancelli.* In some bones, however, such as the ribs, the medullary cavity is absent, and the cancelli take its place throughout the length of the bone; and in some of the smaller bones there is not even the cancellated structure.

In the medullary cavity lies the *medulla,* or marrow, a mass of connective tissue containing an abundance of fat cells. Minute channels, containing blood-vessels, and known as the *Haversian canals,* are found in the walls of the medullary cavity, and the bony substance around these has a peculiar concentric formation, consisting of what are known as *lamellæ.* These contain small openings, *lacunæ,* which are connected with other minute canals, called the *canaliculi.*

Huxley ‡ concisely sums up the general structure of long bones with cartilaginous ends in the following words:—"The bone may be regarded as composed of, *a,* an internal, thick cylinder of vascular medulla; *b*, an external, hollow, thin, cylindrical sheath of vascular periosteum, completed at each end by a plate of articular cartilage; *c*, of a fine, regular, long-meshed, vascular network, which connects the sides of the medullary cylinder with the periosteal sheath of the shaft; *d*, of a coarse irregular vascular meshwork occupying at each end the space between the medullary cylinder and the plate of articular cartilage, and connected with the periosteum of the lateral parts of the articular end; *e*, of the

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* *Zeit. phys. Chem.*, 1895, 20, pp. 361, 362.

† *Skand. Arch. f. Phys.*, 1895, 6, pp. 378-400; quoted by Neumeister, *loc. cit.*, 453.

‡ *Elementary Physiology*, p. 330.

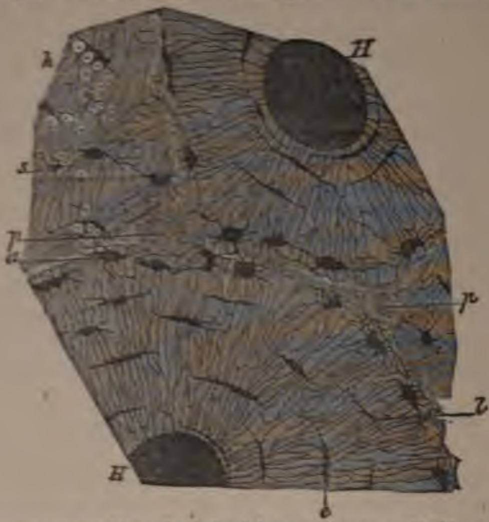

FIG. 9.—Transverse section of shaft of human femur. *H*, Haversian canals; *c*, lacunæ with bone-corpuscles; *a*, lacunæ with recurrent canaliculi; *s*, intermediate lamellæ; *l*, confluent lacunæ. × 300. (*Stirling.*)

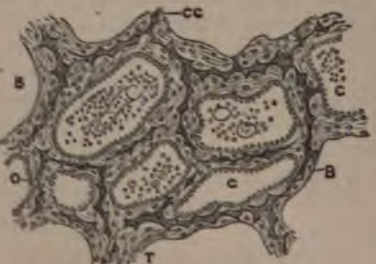

FIG. 10.—Cancellated bone. C, cancellus; cc, calcified cartilage; B, bone; O, osteoblast. (*Stirling.*)

hard, perfect osseous tissue which fills the meshes of these two networks."

Composition.—*Organic Basis.*—The mineral substances of the bone can be removed by treatment with mineral acids, and an organic residue is left which retains the form of the original bone. This substance, formerly known as *ossein*, yields gelatin on treatment with boiling water, like connective tissue proper. It also contains a certain amount of a substance akin to *elastin*.

According to Hoppe-Seyler, from 25 to 26 per cent. of the organic substance of bone consists of collagene, the other constituents amounting to about 8 per cent.

Water.—The quantity of this shows a considerable variation even in the bones from different parts of the same frame. Schodt, for example, found a variation of from 15 to 44 per cent. in the different bones of a dog.

Mineral Basis.—On calcining bone the organic matter is removed, and the mineral framework left behind. This consists principally of calcium phosphate, with calcium carbonate and magnesium phosphate, and small amounts of compounds of potassium, sodium, chlorine, and fluorine. The sodium amounts to about 1 per cent. of the total ash, the potassium to about 0·25 per cent., while the fluorine does not, as a rule, exceed 0·05 per cent., though in exceptional cases it may amount to 0·10. Chlorine is only present in traces.

The following figures, selected from a long table given by Frémy,* show the proportion of mineral matter and some of its constituents in the bones of various animals:—

MINERAL MATTER OF BONE.

Bone.	Per Cent.			
	Total Mineral Matter.	Calcium Phosphate.	Magnesium Phosphate.	Calcium Carbonate.
Rabbit (femur), . .	66·3	58·7	1·1	6·3
Calf (5 months old, femur),	69·1	61·2	1·2	8·4
Cow (old, femur), . .	71·3	62·5	2·7	5·9
Ox (humerus), . .	70·4	61·4	1·7	8·6
Bull (femur), . . .	69·3	59·8	1·5	8·4
Lamb (femur), . .	67·7	60·7	1·5	8·1
Sheep, ...	70·0	62·9	1·3	7·7
Turkey, ...	67·7	63·8	1·2	5·6
Codfish, ...	61·3	55·1	1·3	7·0

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* *Ann. de Chim. et Phys.*, iii. Ser. 42, 47–107.

It is noteworthy that the percentage of the different constituents calculated on the amount of bone-ash show a remarkably constant ratio in the case of different animals. Thus Zalesky [*] gives the following figures :—

	Calcium Phosphate.	Magnesium Phosphate.	Calcium combined with CO_2, Cl, and F.	Carbon Dioxide.
Man, . .	83·89	1·04	7·65	5·73
Ox, . .	86·09	1·02	7·36	6·20
Guinea-pig, .	87·38	1·05	7·03	...
Turkey, . .	85·98	1·36	6·32	5·27

Gabriel,[†] also, gives results in substantial agreement with these :—

	Calcium Oxide.	Phosphoric Acid.	Magnesium Oxide.
Ox, ...	51·28	37·46	1·05
Goose, . . .	51·01	38·19	1·27

The age of an animal appears to cause but little variation in the composition of the mineral matter of the bone, judging by the results of E. Wildt,[‡] who examined the bones of twelve rabbits, varying in age from one day to four years, with the following results :—

Calcium Oxide.	Phosphoric Acid.	Magnesium Oxide.
51·91–52·89	39·78–42·20	0·83–1·38

THE BLOOD.

Quantity in the Body.—Speaking generally, the blood constitutes about one-twelfth of the total weight of the body, but the quantity depends very largely on the condition of the animal, and on the length of time that has elapsed since it has taken food and drink. The amount of blood which is lost when an animal is slaughtered is only a portion of the total quantity, a considerable proportion being retained by the flesh and various organs. According to Fischoeder [§] the quantities which can be collected on slaughtering various animals are on the average: horse,

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[*] Quoted by Neumeister, *Lehrbuch*, p. 456.

[†] *Zeit. phys. Chem.*, 1894, 18, pp. 281, 282.

[‡] Neumeister, *loc. cit.*, p. 457.

[§] *Handbuch der Fleischbeschau*, p. 23.

20 to 25 litres; ox, 15–20 litres; pig, 2–3 litres; sheep, 1 to 1½ litre; and calf, 1 to 1½ litre.

General Characteristics.—The specific gravity of blood shows considerable variation, even in different animals of the same species, the sex, nourishment, and age of the individual all having an influence. The normal limits may be taken as 1·045 and 1·075.

Odour.—Blood has a smell characteristic of the animal from which it was derived. This becomes much more apparent on adding dilute sulphuric acid and gently warming.

Reaction to Litmus.—The chemical reaction is feebly alkaline on account of the presence of sodium carbonate and sodium phosphate. According to Winterstein * the alkalinity of rabbits' blood is equivalent to 0·165 gramme of sodium hydroxide in 100 c.c. The alkalinity of the blood differs no more in different species of animals than in individuals of the same species.

Colour.—This depends chiefly on the quantity of hæmoglobin in the blood. As a rule the blood of carnivorous animals is darker than that of herbivorous animals, and the male has usually darker coloured blood than the female.

Structure.—Blood consists of an almost colourless liquid, the *plasma*, in which are suspended small particles—the colourless and red corpuscles. From the plasma an albuminous substance, *fibrin*, is readily separated, while the clear residual liquid is known as the *serum*. When the coagulation or clotting of blood occurs, a solid mass is formed, consisting of the red corpuscles bound together in a network of fibrin.

Conditions Affecting Coagulation.—The coagulation of blood is accelerated by such conditions as moderate warmth (39°–49° C.), dilution with not more than twice its volume of water, contact with foreign substances, and exposure in shallow dishes to the action of the atmosphere. It is retarded by cold, heat, addition of more than twice its volume of water, imperfect aëration, as in cases of death by suffocation, and by the addition of alkaline salts, strong acids, or alkalies.

THE RED CORPUSCLES.

In man and most mammals these are round, double-concave discs, but in the blood of the camel and llama, and in that of birds, fishes, reptiles, and amphibia, the discs are elliptical. There is a great difference both in their size and number in different animals. As a general rule the blood of reptiles and amphibia contains comparatively few, while they are especially numerous in the blood of carnivora. The accompanying figure represents the appearance of the red and white corpuscles of human blood.

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* *Zeit. phys. Chem.*, 1891, 15, p. 505.

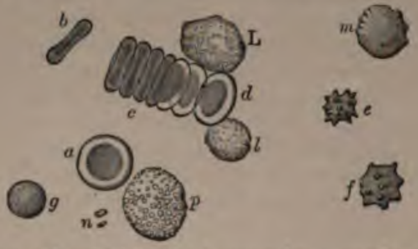

FIG. 11.—Human red and white blood-corpuscles. *a*, red corpuscles, seen on the flat; *b*, in profile; *c*, a rouleau; *d*, three-quarter face; *e*, *f*, crenated; *g*, spherical; *L*, large white corpuscles; *l*, small white corpuscles; *p*, granular leucocyte; *n*, free granulations. × 1000. (*Stirling.*)

According to Bunge,[*] the proportion of blood corpuscles in 100 parts of blood is, in the case of different animals, as follows: horse, 53; pig, 43·5; ox, 35; dog, 35·7. Arouet[*] found human blood to contain on the average 48 per cent.

Colour.—This is due to hæmoglobin, which, in the form of oxyhæmoglobin, composes about 13 per cent. of the total blood, 40 per cent. of the moist corpuscles, and 95 per cent. of their total organic matter. The other constituents of the corpuscles are grouped together under the term *stroma*.

Examined under the microscope, the individual corpuscles are transparent, and have a faint yellowish-green colour.

Oxyhæmoglobin.—This is the colouring matter of the bright red arterial blood. It can be isolated from the *stroma* in a crystalline form. The blood corpuscles are separated from the defibrinated blood by means of centrifugal action, washed free from serum with a solution of sodium chloride, shaken with ether, transferred to a separating funnel with a small quantity of water, and the lower aqueous solution filtered. The filtrate is cooled to zero, mixed with a fourth of its volume of alcohol also at 0° C., and allowed to stand for twenty-four hours at a temperature of from −2° to −10° C. The crystals of oxyhæmoglobin which have deposited are pressed between filter paper, and purified by recrystallization.

Differences in the Oxyhæmoglobin Crystals obtained from the Blood of Different Animals.—The crystals obtained by the method described above vary greatly in chemical composition, crystalline form, and solubility (see fig. 12).

As an instance of the variation in composition the following analysis of different specimens of crystals from horse-blood may be quoted:—

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[*] Quoted by Neumeister, *Phys. Chem.*, p. 559.

	Carbon.	Iron.	Sulphur.
Hoppe-Seyler, . .	54·87	0·47	0·65
Zinoffsky, . . .	51·15	0·34	0·39

The water of crystallization in different varieties of oxyhæmoglobin varies from 3 to 10 per cent.

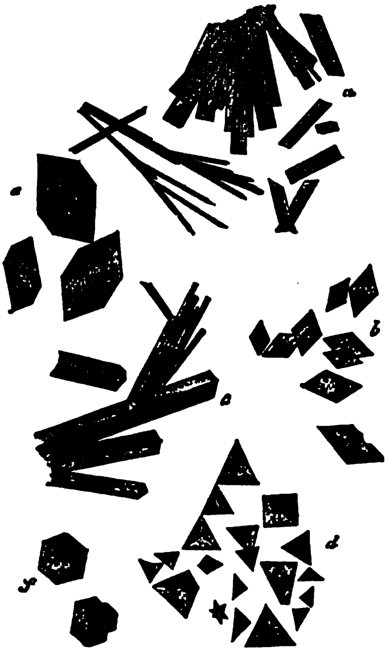

FIG. 12.—Hæmoglobin crystals from Blood. *a*, *b*, human; *c*, cat; *d*, guinea-pig; *e*, hamster; *f*, squirrel. (*Landois and Stirling.*)

As regards crystalline form, the oxyhæmoglobin of human blood is obtained in microscopic rhombic needles, and that of the horse in quadrilateral prisms often several millimetres in length. The blood of the guinea-pig, rat, and many birds yield rhombic tetrahedra, while from that of the squirrel hexagonal plates are deposited.

The difference in solubility is shown by the fact that the crystals are easily prepared from the blood of the guinea-pig, rat, and squirrel, and fairly readily from that of the horse, dog, cat, and mouse, but only with considerable difficulty in the case of the pig and ox.

Identification of Oxyhæmoglobin.—The most characteristic property of oxyhæmoglobin is its absorption spectrum. In a suitable degree of dilution it shows two bands, one at D, and the other, which is broader and less defined, at E. On continued dilution the latter is the first to disappear (*cf.* fig. 15).

Quantitative Estimation of Oxyhæmoglobin.—The amount of oxyhæmoglobin in blood, or in liquids containing blood, can be approximately estimated by determining the amount of iron in the ash. The oxyhæmoglobin from horses' blood contains from 0·34 to 0·47 per cent. of iron, and 0·42 per cent. may be taken as the average amount in dry oxyhæmoglobin in general.

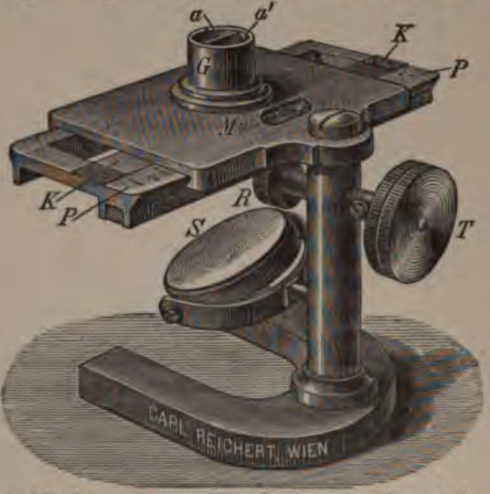

FIG. 13.—Fleischl's hæmometer. *K*, red-coloured wedge of glass moved by *R*; *G*, mixing vessel with two compartments, *a* and *a'*; *M*, table, with hole to read off percentage of hæmoglobin on scale *P*; *T* to move *K*; *S*, mirror of plaster of Paris. (*Landois and Stirling.*)

The method most frequently employed, however, is Hoppe-Seyler's colorimetric process,* or one of its modifications. This consists in diluting a measured quantity of blood with water until it matches the colour of a solution containing a known quantity of pure crystalline oxyhæmoglobin.

The Nessler tubes, and the process recommended by Hehner † for the determination of ammonia in water, are well adapted for this colorimetric process.

G. Oliver ‡ advocates the use of Lovibond's tintometer and standard colour glasses, and, according to Halliburton, ‡ this gives a better result with diluted blood than any other colorimetric process.

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* *Zeit. physiol. Chem.*, 1892, xvi. p. 505.

† *Analyst*, 1877, ii. p. 180.

‡ *Kirk's Physiology*, p. 597.

Fleischl's hæmometer, which is one of those most frequently used, is illustrated in fig. 13.

Reduction of Oxyhæmoglobin.—The oxygen in oxyhæmoglobin is only feebly combined, and is completely liberated *in vacuo*. It is known as 'respiratory oxygen,' and the iron in the proteid molecule probably plays some part in its addition. Besides being reduced in a vacuum, oxyhæmoglobin is also converted into hæmoglobin by passing a current of an inert gas such as hydrogen, carbon dioxide, or nitrogen through a solution of it, by the addition of reducing agents such as ammonium sulphide, and by the products of putrefaction.

'*Pseudo-Hæmoglobin.*'—Certain reducing agents, such as potassium hydro-sulphide, only cause a partial reduction of oxyhæmoglobin, and an intermediate product is formed with the same spectrum as the completely reduced oxyhæmoglobin, but still containing some oxygen.

Hæmoglobin.—This is obtained by the complete reduction of oxyhæmoglobin, and is the dark purple colouring matter of the venous blood. It combines energetically with oxygen and carbon monoxide. Its spectrum is shown in fig. 15.

Hæmatin.—On heating an aqueous solution of oxyhæmoglobin to about 80° C. it undergoes decomposition, and an amorphous precipitate is deposited, which has a composition corresponding to the formula $C_{32}H_{32}N_4O_4Fe$. Hæmatin has a bluish-black metallic lustre, and can be heated to 180° C. without decomposition.

Hæmin.—This is the hydrochloric acid compound of hæmatin anhydride, and has the formula $C_{32}H_{30}N_4O_3Fe.HCl$. It is precipitated in characteristic crystals on heating a solution of oxyhæmoglobin in glacial acetic acid containing a little sodium chloride. The crystals are minute rhombic plates with a bluish-black metallic lustre. They are insoluble in water, alcohol, and ether, slightly soluble in acetic acid and dilute mineral acids, and easily soluble in alkaline liquids and acidified alcohol. The difference in the form of hæmin crystals from different kinds of blood is seen in fig. 14.

Hæmatin Acid.—This has recently been prepared by Kuster * by oxidizing hæmatin dissolved in acetic acid. It is a crystalline, dibasic acid, and has the formula $C_8H_{10}O_5$.

Hæmo-chromogen, or reduced hæmatin, is produced by the action of acids and alkalies on hæmoglobin in the absence of oxygen. In alkaline solution it rapidly absorbs oxygen from the air, changing to hæmatin. In acid solution it gradually loses its iron, and becomes converted into hæmato-porphyrin.

Hæmato-porphyrin.—When hæmatin or hæmin crystals are treated with concentrated sulphuric acid, fuming hydrochloric acid, or acetic acid and hydrobromic acid, the iron is completely

* *Berichte*, 1897, p. 105.

split off, and, on dilution, a colouring matter is obtained. This has the composition $C_{32}H_{36}N_4O_6$, and was named hæmato-porphyrin by Hoppe-Seyler.

Methæmoglobin.—By acting on the colouring matter of blood with oxidizing agents, such as potassium permanganate, hydrogen peroxide, or ozone, the oxyhæmoglobin undergoes a molecular change, and an isomeric compound with a distinctive spectrum is formed. It can be reconverted into oxyhæmoglobin by dissolving it in dilute sodium hydroxide solution, and adding a reducing agent such as ammonium sulphide.

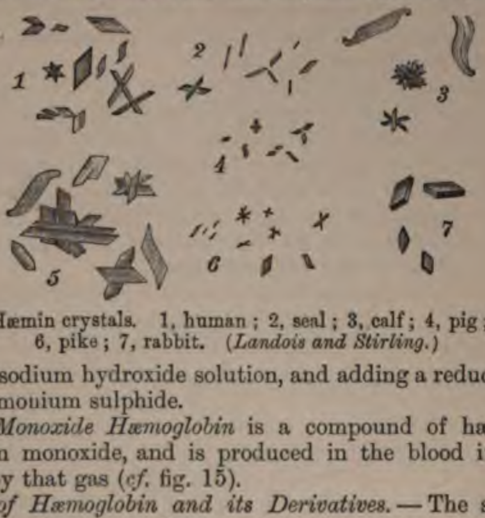

Fig. 14.—Hæmin crystals. 1, human; 2, seal; 3, calf; 4, pig; 5, lamb; 6, pike; 7, rabbit. (*Landois and Stirling.*)

Carbon Monoxide Hæmoglobin is a compound of hæmoglobin with carbon monoxide, and is produced in the blood in cases of poisoning by that gas (*cf.* fig. 15).

Spectra of Hæmoglobin and its Derivatives.—The spectra of hæmoglobin and of some of its most characteristic compounds are shown in the accompanying figure (fig. 15).

THE WHITE CORPUSCLES.

The white corpuscles, or *leucocytes*, are present in the blood in much smaller quantity than the red corpuscles, the normal ratio being about 1 : 350. They are also considerably larger, being usually about $\frac{1}{2500}$ of an inch in diameter. In form they are irregular, and are continually altering their shape after the manner of the *amœba*. They are composed of a granular substance of a protoplasmic nature containing a rounded *nucleus*, which can readily be made visible by treating the white corpuscles with very dilute acetic acid. This nucleus assumes a darker colour than the rest of the corpuscle when stained with carmine. Fig. 16 represents the appearance of the white corpuscles when treated with different reagents.

OTHER SMALL BODIES IN BLOOD.

In addition to the corpuscles, blood contains small irregularly shaped bodies containing protoplasm, to which the name of *blood platelets* or '*hæmatoblasts*' has been given. There also occur here and there small particles, some of which contain a brown or black pigment. These are about five times the size of the red corpuscles, and possibly have their origin in the spleen. The colourless particles which are met with in the blood are probably fragments of broken-up white corpuscles.

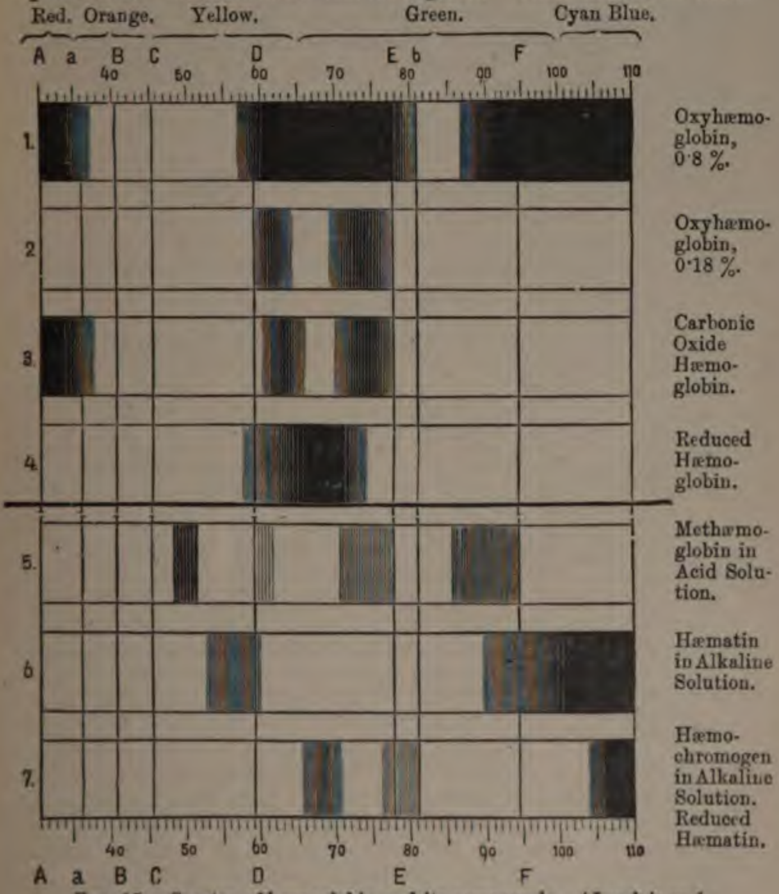

FIG. 15.—Spectra of hæmoglobin and its compounds. (*Landois and Stirling.*)

THE BLOOD PLASMA.

This contains about 8 per cent. of solid matter, chiefly of a proteid nature. The amount of inorganic matter is about 0·75 per cent.

Proteids of the Plasma.—The albuminous substances contained in blood plasma are chiefly globulins. Serum albumin is also present in small proportion, the ratio between it and the globulins varying in different species of animals. The plasma of cold-blooded animals contains the least serum albumin.

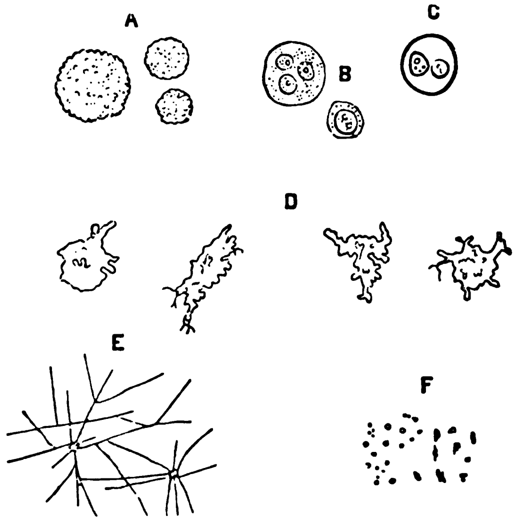

Fig. 16.—White corpuscles under different reagents. *A*, human white blood corpuscles without any reagent; *B*, after the action of water; *C*, after acetic acid; *D*, frog's corpuscles; changes of shape due to amœboid movement; *E*, fibrils of fibrin from coagulated blood; *F*, elementary granules. (*Landois and Stirling.*)

Metaglobulin or Fibrinogen.—This is the most important of the proteid constituents. It can be separated from the globulins and serum albumin by adding sodium chloride to its solution. When the amount of sodium chloride reaches 16 per cent., the fibrinogen is precipitated, whilst the globulins remain in solution until the liquid has been saturated with salt.

On heating fibrinogen in aqueous solution to from 56° to 60° C., it is decomposed into two globulins, one of which is precipitated, while the other remains in solution until the temperature reaches 65° C.

After removing the sodium chloride by means of dialysis, fibrinogen is obtained in white flakes, which readily agglomerate into an elastic mass. When left in water it undergoes an alteration and becomes insoluble in dilute salt solutions.

'Thrombin' or 'Fibrin Ferment.'—According to A. Schmidt * and others the coagulation of fibrinogen is brought about by an enzyme, the so-called 'thrombin' or 'fibrin ferment,' which is derived from a substance of a similar nature, '*prothrombin*,' contained in the corpuscles of the blood, more especially the white corpuscles. It can be obtained from the defibrinated blood or from the blood serum by leaving the liquid in contact with strong alcohol for several months, and extracting the air-dried deposit with a little water. This aqueous extract contains the active enzyme, and rapidly causes a solution of fibrinogen to coagulate in the presence of a small quantity of calcium chloride.

Fibrin.—On coagulation fibrinogen is decomposed into two proteid bodies—*fibrin* and *fibrin globulin*. The quantity of fibrin obtained from blood only amounts to from 0·1 to 0·4 per cent., although from its voluminous nature it appears considerably more. When deposited during the coagulation of blood it is very impure, being mixed with serum globulin and constituents of the white corpuscles. The pure substance can be prepared by treating a solution of pure fibrinogen containing calcium chloride with the fibrin ferment. Pure fibrin is insoluble in water and (at first) in neutral saline liquids, but is completely soluble in dilute acids and alkalies after remaining in contact with them for some days, and in dilute salt solutions after some weeks.

Fibrin Globulin.—This substance is formed together with fibrin during the coagulation of fibrinogen. It is also produced together with another proteid on heating fibrinogen to 56–60° C. On evaporating its aqueous solution fibrin globulin is converted into a substance of an albumose character.

Paraglobulin or Serum Globulin.—When blood coagulates (with deposition of the altered fibrinogen) this proteid is found in an unaltered state in the serum. A solution of paraglobulin in a 10 per cent. solution of sodium chloride coagulates at 75° C. It can be precipitated by adding to its solution an equal volume of a saturated solution of ammonium sulphate.

Serum Albumin.—This is also found in the serum after coagulation of the blood and can be isolated by precipitating the globulins by saturating the liquid with magnesium sulphate at 30° C., and then adding dilute acetic acid to the filtrate. Pure serum albumin coagulates from its aqueous solution at 50° C., but when salts are also present the coagulation temperature is considerably raised.

Yellow Colouring Matters of Blood Serum.—The faint yellow colour of the blood serum of man and most animals is due to the presence of a dissolved colouring matter (lipochrome), which can be extracted by means of amyl alcohol. This colour is much more pronounced in certain animals than in others. It is well marked, for instance, in the serum of the ox, pigeon, hen, and tortoise, whilst rabbits' serum is almost colourless. Under the name 'lipochrome' are classified various non-nitrogenous animal colouring matters, the constitution of which has not been determined.

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* Neumeister, *loc. cit.*, p. 594.

Hammarsten [*] found that the blood serum of the horse always contained a small quantity of *bilirubin*, one of the colouring substances of the bile. The amount of this appears to vary in different horses.

Fat.—This may amount to as much as 1 per cent. in the case of animals whose food has contained a large amount of fat. It can be extracted from the serum with ether.

Cholesterin and lecithin are invariably present in small proportions.

Glucose.—This is a constant constituent in varying quantity in the blood serum of different animals, but it never appears to exceed 0·2 per cent. of the original blood. Other reducing bodies, including gums, are also present in traces. Traces of sarcolactic acid, kreatine, uric acid, and urea are also present.

Inorganic Constituents of Plasma.—These are found partly in combination with the proteids and partly in the free state. Bunge [†] obtained the following mean results in his examination of the blood serum of the horse, ox and pig:—Potassium oxide, 0·026; sodium oxide, 0·435; calcium oxide, 0·013; magnesium oxide, 0·004; chlorine, 0·369; phosphoric acid, 0·022; total, 0·869 per cent.

The sodium chloride is in the free state, and not in combination with the proteids. A considerable proportion of the sodium is in the form of sodium bicarbonate. In addition to these salts traces of fluorine compounds have also been found.

Gases in Blood.—These are oxygen, carbon dioxide, and nitrogen. Arterial blood contains more oxygen than venous blood, while the latter is richer in carbon dioxide. Pflüger [‡] obtained from the arterial blood of a dog, 21 per cent. by volume of oxygen, and from the venous blood 12 per cent. In the case of the carbon dioxide the respective quantities were 38 and 46 per cent. by volume. There is not this difference to be observed in the amount of nitrogen, which, in each kind of blood, is about 2 per cent.

The carbon dioxide is present partly in the form of sodium bicarbonate, and the remainder is probably loosely combined with some of the proteid substances. Practically the whole of the oxygen is in chemical combination with the hæmoglobin of the red corpuscles.

[*] Neumeister, *loc. cit.*, p. 585.

[†] *Zeit. Biol.*, 1876, 12, p. 191.

[‡] Neumeister, *loc. cit.*, p. 600.

ULTIMATE COMPOSITION OF BLOOD.

According to the analysis of Playfair and Boeckmann, the dried blood of the ox has the following elementary composition:—Carbon, 57·9; hydrogen, 7·1; nitrogen, 17·4; oxygen, 19·2; ash, 4·4 per cent. This would correspond to the formula $C_{45}H_{39}N_{16}O_{15}$, which is identical with that found by the same chemists for flesh.

PROXIMATE COMPOSITION OF BLOOD.

E. Abderhalden* gives the following table of the quantity of the various constituents in the blood of the ox and horse.

COMPOSITION OF THE BLOOD OF THE OX AND HORSE.

	Grammes in 1000 Grammes.					
	Ox.			Horse.		
	Blood.	Blood Serum.	Blood Corpuscles.	Blood.	Blood Serum.	Blood Corpuscles.
Water, . .	808·9	913·64	591·858	749·02	902·05	613·15
Solid Matter, .	191·1	86·36	408·141	250·98	97·95	386·84
Hæmoglobin, .	82·0	...	251·92	166·9	...	315·08
Albumin, . .	90·9	72·5	129·02	69·7	84·24	56·78
Sugar, . .	0·7	1·05	...	0·526	1·176	...
Cholesterin, .	1·935	1·238	3·379	0·346	0·298	0·388
Lecithin, . .	2·349	1·675	3·748	2·913	1·720	3·973
Fat, . . .	0·567	0·926	...	0·611	1·300	...
Phosphoric Acid in Nucleïns, .	0·0267	0·0133	0·0546	0·060	0·020	0·095
Sodium Oxide, .	3·635	4·312	2·2322	2·091	4·434	...
Potassium Oxide,	1·407	0·255	0·722	2·738	0·163	4·935
Iron Oxide, .	0·544	...	1·671	0·828	...	1·563
Calcium Oxide, .	0·069	0·1194	...	0·051	0·1113	...
Magnesium Oxide,	0·0356	0·0446	0·0172	0·064	0·045	0·0809
Chlorine, . .	3·079	3·69	1·8129	2·785	3·726	1·949
Phosphoric Acid,	0·4038	0·244	0·7348	1·120	0·240	1·901
Phosphoric Acid in inorganic combination, .	0·1711	0·0847	0·3502	0·806	0·0715	1·458

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* *Zeit. physiol. Chem.*, 1897, **23**, p. 521.

IDENTIFICATION OF BLOOD IN STAINS, ETC.

This belongs rather to the domain of forensic chemistry than to the subjects treated of in the present work, and hence no exhaustive description of the methods which have been recommended for the purpose is attempted here. The following details may, however, be found serviceable.

*Alteration of Dried Blood on Heating.**—When dried blood is heated for an hour at 100° C., it still remains soluble in water, cold saturated solutions of borax, concentrated solutions of potassium cyanide, dilute sodium hydroxide solution, ammonium hydroxide, acidulated alcohol, and glacial acetic acid.

After being heated for an hour at 120° C., it becomes insoluble in water, and less soluble in the other liquids, with the exception of sodium hydroxide solution, and acetic acid, in which it dissolves as readily as before.

After an hour at 140° to 180° C., it is only slightly soluble in ammonium hydroxide, but more so in sodium hydroxide solution and glacial acetic acid, which must therefore be regarded as the most suitable solvents.

Preparation of Hæmin Crystals.—This is generally looked upon as the most characteristic test for blood. By using potassium iodide instead of sodium chloride, Strzyzowski * was able to detect as little as 0·000025 gramme. In his method a trace of the substance under examination is mixed with a drop of a 0·2 per cent. solution of aqueous potassium iodide on a glass slide, and after the liquid has evaporated, a cover glass is placed over the residue, and a little glacial acetic acid introduced. The slide is gently heated until the acetic acid commences to boil, and when cold is examined under the microscope.

Spectroscopical Examination.—This often furnishes corroborative evidence. The absorption spectra of some of the colouring substances, obtained from blood, are shown on p. 39.

Detection of Blood in the Presence of Iron.—It is often impossible to obtain hæmin crystals from blood which has become insoluble from being left in contact with iron. In such cases Gantter † recommends the use of hydrogen peroxide as a reagent. A drop of the solution, or a fragment of the insoluble substance moistened with water, is made feebly alkaline, and a drop of hydrogen peroxide added. If the slightest trace of blood be present, numerous bubbles of oxygen are liberated, which, in a short time, coalesce into a white scum.

Unfortunately, other animal fluids (*e.g.* pus) behave in a similar manner, so that a positive result is not absolutely conclusive. Still the test is of value as confirmatory evidence.

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* *Zeit. anal. Chem.*, 1898, p. 467.

† *Ibid.*, 1895, pp. 159, 160.

THE BLOOD OF INVERTEBRATE ANIMALS.

Hæmolymph.—In worms and the majority of molluscs, the liquid which corresponds to the blood in higher animals, fulfils the functions of blood and lymph, and has hence received the name of '*hæmolymph.*' It is a liquid rich in albuminous substances, and in many cases shows signs of fibrin coagulation. It contains white corpuscles (leucocytes), and in some few instances, red corpuscles, but the latter are rare. Instead of red corpuscles, free oxyhæmoglobin is not unfrequently found in solution, its function being probably of a respiratory nature, and the violet or purple colouring matter in the hæmolymph of some invertebrata appears to play a similar part.

Oxyhæmocyanin.—In certain arthropoda and molluscs (*e.g.* the crab, oyster, and snail), the hæmolymph has a bright blue colour, due to the presence of an albuminous colouring matter, which takes the place of the oxyhæmoglobin in red blood, but which contains copper instead of iron. This pigment, known as '*oxyhæmocyanin*,' can be partially separated from the hæmolymph by dialysis, but it readily redissolves in a dilute solution of sodium chloride. It coagulates at 68° to 69° C., and, like the globulins, can be precipitated by saturating its solution with magnesium sulphate or sodium chloride.

When acted upon by acids, it is decomposed into albumin, and a colouring substance which contains a large proportion of copper, and corresponds to *hæmatin.* When the respiratory oxygen is withdrawn from it *in vacuo*, or by means of reducing agents, a colourless compound (*hæmocyanin*) is left, which rapidly becomes blue again on exposure to the air. Neither of these compounds appears to have a very definite absorption spectrum.

Other pigments, of the nature of lipochromes, have also been found in hæmolymph of various origin, but these have not been proved to have any definite respiratory functions.

CHAPTER III.

THE FLESH OF DIFFERENT ANIMALS.

THERE is no more difficult problem in analytical chemistry than the detection of one kind of flesh when mixed with another. It is true that each has its own characteristic odour, but the substance producing this is probably present in too minute a quantity to be isolated from the flesh or separated from a mixture of such bodies. Then, too, the chemical differences which have been recorded by various observers are in most cases incapable of giving reliable data, owing to the variation in the composition of the flesh of different animals of the same species, and the similarity of that of animals of different species.

In some cases an examination of the fat of the adipose tissue connected with the muscle or of the fat within the muscular tissue itself may be of service, as, for instance, in the detection of horseflesh in meat preparations,* and for that reason the chemical and physical constants of the fat of different animals are given at length in the following pages.

Sometimes the flesh contains a considerable proportion of a given constituent, which is either absent altogether or only present in smaller amounts in other kinds of flesh, as, for example, isokreatinine in the flesh of the haddock, betaine in the mussel, and glycogen in horse flesh. In such cases a quantitative determination of the substance may give an approximate idea of the amount of the original flesh. It is only, however, in the case of horse-flesh that such a method has as yet been worked out with any degree of success.

C. Virchow † made experiments to determine whether there was a difference in the amount of soluble extractives in different kinds of flesh. After removing all visible fat, the finely divided flesh was extracted with water at 45° C., the extract boiled, filtered from the coagulated albumin, an aliquot part of the filtrate evaporated, and the residue dried and weighed. His results showed that

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* *Cf.* p. 141.

† *R. Virchow's Archiv*, 1881, p. 543.

COMPOSITION OF THE FLESH OF DOMESTIC ANIMALS.

		Per Cent.					Per Cent. On the Dry Substance.			
Animal.	Condition.	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.	Nitrogenous Substances.	Fat.	Nitrogen.	No. of Analyses.
Ox,	very fat,	53·05	16·75	29·28	...	0·92	35·46	62·36	5·67	11
	medium,	72·03	20·96	5·41	0·46	1·14	74·95	19·53	12·00	42
	lean,	76·37	20·71	1·74	...	1·18	87·64	7·37	14·02	13
Cow,	fat,	70·96	19·86	7·70	0·41	1·07	69·56	25·83	11·13	...
	lean,	76·35	20·54	1·78	...	1·32	86·95	7·25	13·92	6
Calf,	fat,	72·31	18·88	7·41	0·07	1·33	68·87	26·04	11·02	9
	lean,	78·82	19·86	0·82	...	0·50	93·89	3·87	15·01	4
Sheep,	fat,	53·31	16·62	28·61	...	0·93	35·59	61·28	5·70	9
	lean,	75·99	17·11	5·77	...	1·33	71·33	23·70	11·43	8
Pig,	fat,	47·40	14·54	37·34	...	0·72	28·16	70·44	4·50	5
	lean,	72·57	20·25	6·81	...	1·10	73·87	24·56	11·82	10
Horse,	(minimum),	61·39	18·90	0·50	...	0·97	56·10	2·02	8·98	12
	(maximum),	79·30	23·30	15·64	1·05	1·12	93·95	40·51	14·53	
	(mean),	74·20	21·70	0·46	0·46	1·01	85·69	8·46	13·71	

there was no appreciable difference in the total amount of extractives yielded by the muscular fibre of different species, or of different animals of the same species, whatever their age, condition, or food.

In the case of the ox his mean results were:—

	Ox (healthy). Fat.	Ox (healthy). Lean.	Ox (diseased).	Calf.	
Water, ...	76·68	76·25	77·47	77·61	per cent.
Extractives, ...	3·73	3·53	3·87	3·82	,,
,, calculated on dry substance, .	15·78	15·09	17·19	17·22	,,

In the description given in this chapter of the varieties of flesh more commonly used as food, a classification into four groups has been adopted for the purpose of convenience:—(1) Domestic animals; (2) Game and birds; (3) Vertebrate fish; (4) Invertebrata.

The word 'flesh' is here chiefly used in its colloquial sense of fat and lean meat.

THE FLESH OF DOMESTIC ANIMALS.

There is, in general, a marked distinction between the flesh of the common domestic animals and birds and of 'game,' the muscular tissue of the former being of a coarser texture, and undergoing putrefactive decomposition more readily than that of the latter. This difference must be chiefly attributed to the difference in food, environment, and habits of life, for when a domestic animal is placed under the same conditions as a wild one its flesh in the course of future generations assumes the finer texture and other characteristics of 'game,' an instance of which is seen in the case of Welsh mountain mutton.

The sex of an animal often has an influence on the physical characteristics of its flesh, and, as a rule, the flesh of the female is more tender, but has less flavour, than that of the male.

The table on p. 47, compiled from those of König,* gives the mean results of the analyses of different chemists.

The subjoined table on p. 49 by Strohmer † gives an idea of the comparative composition of the chief animals in this group regarded from another point of view.

The average composition of commercial lean meat, freed from bone and all visible fat, is given by Voit as:—Water, 75·9; proteids, 18·4; collagenous substance, 1·6; fat, 0·9; extractives, 1·9; and ash, 1·3 per cent.

* *Nahr. u. Genussmittel*, ii. 110.

† *Die Ernahrung des Menschen*, p. 112.

	Fat Calf.	Medium Ox.	Fat Ox.	Fat Lamb.	Lean Sheep.	Fat Sheep.	Lean Pig.	Fat Pig.
Age of Animal, years,	¾	4	4	½	1	1¼	?	?
Living Weight, kilos.	258	1232	1419	84	97	107	93	185
Contained per cent.—								
Bone, ...	12·4	11·4	10·4	8·1	9·5	7·0	8·3	5·6
Muscular flesh, . .	45·5	47·9	40·2	36·9	37·5	29·8	47·6	37·3
Fat, ...	11·0	12·7	25·8	23·7	14·8	32·4	20·0	39·4
Entrails, skin, etc.,	31·1	28·0	23·6	31·3	38·2	30·8	24·1	17·7
Butcher's refuse, .	37·9	35·2	33·8	40·2	46·7	42·5	26·3	17·2
Fit for food, . . .	62·1	64·8	66·2	59·8	53·3	57·5	73·7	82·8

BEEF.

Characteristics.—The muscular tissue of the ox has a somewhat closer texture than that of the other animals in the preceding table, and retains more of the blood. In certain parts the flesh is nearly free from visible fat, in others the fat is intermingled with the lean, giving a mottled appearance. The connective tissue of an animal in good condition glistens on exposure to the air, and is fairly moist, though no water should exude from it.

The proportion of muscular tissue, fat, etc., contained in an ox are shown in the following table of Lawes and Gilbert:—

		Per cent.			
Condition.	Age.	Bones.	Muscle.	Fat.	Skin, etc.
Moderately fat, . . .	3 years	11·4	47·9	12·7	28·0
Fat, ...	„	10·4	40·2	25·8	23·6

Influence of Sex.—The flesh of the cow is more tender than that of the ox, but has somewhat less flavour. That of the bull has a rank, strong taste, and in consequence bull-beef may only be exposed for sale in this country with a plain notification as to its nature.*

In general it may be stated that the flesh of the male uncastrated animal has a more pronounced taste and smell than that of the female or castrated male.

Composition.—According to the analyses of Playfair and Boeckmann,† beef has the following ultimate composition:—

	Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Ash.
Playfair, . .	51·83	7·57	15·01	21·37	4·23
Boeckmann, .	51·89	7·59	15·05	21·24	4·23

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* Public Health Act, 1875, § 261.

† Liebig, *Organic Chemistry*, p. 314.

Almen * found its proximate percentage composition to be:—Water, 76·76; solid matter, 23·24; proteids, 17·88; fat, 2·24; insoluble salts, 0·65; and soluble salts, 0·48.

As an instance of the variation in the composition of the flesh from different parts of the same animal, the following results may be quoted from König:—

Flesh of Fat Ox from	Per Cent.				Per Cent. Calculated on Dry Substance.		
	Water.	Nitrogenous Substances.	Fat.	Ash.	Nitrogenous Substances.	Fat.	Nitrogen.
Neck, . .	73·5	19·5	5·8	1·2	73·58	21·89	11·77
Shoulder,	50·5	14·5	34·0	1·0	39·29	68·69	4·68

Digestibility.—Judging by the results of artificial digestion experiments, the muscular tissue of the ox is the most digestible of all the kinds of flesh ordinarily eaten. †

The Fat.—Beef-fat shows considerable variation in colour according to the age and food of the animals. In young bulls it is whiter than in cows and bullocks, and the fat of animals fed on oil-cake is much yellower than that of animals fed on grass or corn. The fat of certain breeds of cattle, notably those of Jersey and Guernsey, is of a deep yellow colour.

In composition beef-fat consists almost entirely of the glycerides of stearic, palmitic, and oleic acids, and is much more constant in its consistency and composition than the fat of the sheep and pig, although the variation in the fat from different parts of the body is considerable.

Lewkowitsch ‡ gives the ratio of stearin to palmitin as about 1:1, a statement which is borne out by Hehner and Mitchell,§ who found a specimen of fresh beef 'stearin' (*i.e.* fat from which the liquid portion had been almost entirely removed, the iodine value being only 2) to contain 50 per cent. of the glyceride of stearic acid, the remainder being, presumably, palmitin.

Beef-fat has a characteristic odour, and on crystallization from ether gives fan-shaped bunches of needle-shaped crystals consisting of a mixture of palmitin and stearin. This has been largely employed as a test for the presence of beef-fat in lard. ||

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* Falck, *Das Fleisch*, p. 346.

† *Cf.* p. 87.

‡ *Oils, Fats, and Waxes*, 1895, p. 482.

§ *Analyst*, 1896, p. 328.

|| *Cf.* p. 91.

Lewkowitsch* gives the following Table (see p. 52), by Leopold Mayer, of the composition and constants of the fat taken from different parts of the body of a Hungarian ox, three years old.

The iodine value of beef-fat varies, according to different observers, from 35·4 (Filsinger) to 44 (Wilson), and that of the liquid fatty acids from 92 to 92·5 (Wallenstein and Finck).

Farnsteiner has found traces of linolic acid and of linolenic acid in ox-tallow (p. 101).

VEAL.

Characteristics.—The flesh of the calf is of a paler colour and less consistent than beef. It was formerly a general practice to increase the paleness by bleeding the animal before death—a custom which has happily fallen into disuse in this country.†

In Germany it is illegal to kill a calf for food at a younger age than ten or twelve days, while a month is the time prescribed in a corresponding Austrian regulation.

The muscular tissue of an embryo or of a newly-born calf is watery, and the fat has a soapy appearance and a distinctive odour.

According to Walley † the flesh of a young calf closely resembles that of a dog, and the same authority states that veal is occasionally substituted for chicken in certain food pastes.

Lawes and Gilbert found a fat calf six months old to have the following percentage composition: bone, 12·4; muscle, 45·5; fat, 11·0; skin, etc., 31·1.

The general composition of calf's flesh is given in the table on page 47, and the fat has practically the same chemical characteristics as beef-fat.

Veal contains much less iron and alkali salts than beef, but, on the other hand, is richer in connective tissue.

According to Staffel ‡ the ash, after deducting the sodium chloride, has the following percentage composition: potassium phosphate, 68·05; sodium phosphate, 5·66; calcium phosphate, 3·72; magnesium phosphate, 6·21; free phosphoric acid, 15·10; ferric oxide, 0·30; and silica, 0·92.

MUTTON.

Characteristics.—The muscular tissue of the sheep differs from beef in its colour and in being less firm in texture. The flesh of an old ram, however, has a marked colour, and is firm and tough.

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* *Loc. cit.*, p. 480.

† Walley, *Meat Inspection*, p. 15.

‡ Liebig, *Letters on Chemistry*.

CHARACTERISTICS OF FAT FROM DIFFERENT PARTS OF AN OX.

Fat from	Fat.				Fatty Acids.				
	Melting Point. °C.	Solidifying Point. °C.	Saponification Value.	Hehner Value.	Solidifying Point. °C.	Melting Point. °C.	Saponification Value.	'Stearic Acid' solidifying 54·8° C.	'Oleic Acid' solidifying 5·4° C.
Intestines, . .	50·0	35·0	196·2	95·7	44·6	47·5	201·6	51·7	48·3
Lungs, . .	49·3	33·0	196·4	95·4	44·4	47·3	204·1	51·1	48·9
Caul, . .	49·6	34·5	198·0	95·8	43·8	47·1	203·0	49·0	51·0
Heart, . .	49·5	36·0	196·2	96·0	43·4	46·4	200·3	47·5	52·5
Neck, . .	47·1	31·0	196·8	95·9	40·4	43·9	203·6	38·2	61·8
Groins, . .	42·5	35·0	198·3	95·4	38·6	41·1	199·6	33·4	66·8

The fat is whiter, and both fat and lean have a more distinctive odour than that of beef.

Walley states that goats' flesh, which is much darker, is occasionally substituted for mutton, and that of the kid for lamb.

Composition.—The flesh, like that of the ox, is of different composition in different parts of the body, as is shown in the following results given by König:—

Flesh from Sheep.	Per Cent.				Per Cent. Calculated on Dry Substance.		
	Water.	Nitrogenous Substances.	Fat.	Ash.	Nitrogenous Substances.	Fat.	Nitrogen.
Hindquarter,	41·97	14·39	43·47	0·66	24·80	74·91	3·97
Breast, . .	41·39	15·45	42·07	1·03	26·36	71·78	4·22
Shoulder, .	60·38	14·57	23·62	0·85	36·77	59·62	5·80

'Braxy Mutton.' *—It is a very common practice in Scotland for the carcasses of sheep which have died from what is known as the 'braxy' to be cured and smoked and used for food, although quite unsaleable in the market. The shepherds distinguish three kinds of braxy, viz., *turnip braxy*, *wet braxy*, and *red braxy*. Turnip braxy is a disease of the blood brought on when the sheep have been fed upon an excess of roots, especially turnips. It comes on very suddenly, only lasts for a few hours, and is very fatal. There is a rapid infusion of the blood serum, containing an excess of albuminous matter, into the connective tissues, the result of which is that the flesh after death feels adhesive to the touch and rapidly glazes over. The term '*wet braxy*' is used in a very loose sense, "practically including all dropsical conditions, whether arising from diseases of the blood such as anæmia, or from organic disease." Under the name of '*red braxy*' are grouped all conditions in which the flesh and internal organs are markedly discoloured. There is obviously considerable risk in eating such flesh, especially in the case of 'red braxy,' where the term is so indefinite in its application that the flesh of an animal which has died of anthrax might very easily come under the same heading.

The Fat.—Sheep's fat varies very greatly in consistency and colour according to the part of the body from which it is derived. The harder fat (tallow) is found principally in the back, neck, and kidneys, whereas that from the ham and breast is fluid or nearly so at the ordinary temperature.

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* Walley, *Meat Inspection*.

Hehner and Mitchell* found that the consistency of the fat was largely dependent on the percentage of stearic acid it contained. They obtained the following results with the fat from different parts of the same sheep.†

STEARIC ACID IN SHEEP'S FAT.

Fat from	In the Total Fatty Acids.	In the Saturated Fatty Acids (calculated).
	Per cent.	Per cent.
Back,	24·8	78
Neck,	16·4	36
Breast,	1·0	3
Ham,	none	none
Kidney,	26·5	58

Mutton fat has a characteristic odour, and turns rancid more readily than beef fat.

When crystallized from ether it deposits crystals closely resembling those obtained from beef stearin † :—

CONSTANTS OF SHEEP'S FAT.

Description.	Melting Point.		Solidifying Point. F. Acids.	Iodine Value. Fat.	Saponification Value. = Mgrms. KOH.	Authority.
	Fat.	F. Acids.				
	°C.	°C.	°C.			
Two Sheep.						**Moser, quoted by Lewkowitsch, *Oils, Fats, and Waxes*, p. 485.**
Fat from kidneys, . . .	54–55	56·2–56·5	51·9–51·9	..	194·8–195·2	
„ caul and intestines,	52 0–52·9	54·9–55·8	50·4–50·6	..	194·6–194·8	
„ adipose tissue, .	49·5–49·6	50·7–51·1	43·7–46·2	..	194·2 194·4	
Fat of Scotch sheep, 18 months old.						**Hehner and Mitchell, *Analyst*, 1896, pp. 327, 328.**
From back, ...	..	41·4	..	61·3	..	
„ neck, ...	..	42·2	..	48·6	..	
„ breast, ...	..	33·8	..	58·2	..	
„ ham, ...	..	40·8	..	50·6		
„ kidney, ...	..	45·6	..	48·16	**Mol. Equiv. F. Acids of Kidney Fat, 276·1.**	

PORK.

Characteristics.—The flesh of pigs has always a distinctive odour, which is very marked in the case of old boars. In the young animals the flesh is very pale and soft, but becomes darker and firmer with age.

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* *Analyst*, 1896, p. 327.

† *Cf.* p. 99.

According to König * the food of the animal has a marked influence on the flavour of its flesh. Thus, pigs fed on potatoes in excess have a very white and flavourless flesh, whilst the flesh of animals whose food has consisted largely of beech-nuts has an oily taste.

The muscular fibre of the pig turns grey on treatment with alcoholic potassium hydroxide, a characteristic which distinguishes it from beef and horse flesh. †

The well-recognized indigestibility of pork is possibly due to the larger amount of fat which it usually contains.

Strohmer ‡ gives the following analyses of flesh from different parts of a fat pig:—

Flesh from	Water.	Nitrogenous Substances.	Fat.	Ash.
Leg, ...	48·71	15·98	34·62	0·69
Neck, . . .	54·63	16·58	28·03	0·76
Ribs, ...	43·44	13·37	42·59	0·60
Shoulder, . . .	40·27	12·55	46·71	0·47
Head, . . .	49·96	14·23	34·74	1·07

The Fat.—Pigs' fat is composed for the most part of the triglycerides of palmitic, stearic, and oleic acids, with, at all events in some cases, small quantities of linolic acid. It is probable that the more fluid fat, such as that from the ham and the breast, contains more of the latter fatty acid than does the more solid fat, such as that from the flare. If so, this would partly account for the higher iodine value of certain American lards, in which the fat is often of a much more mixed character than in European lards, which are prepared for the most part from the fat of the kidney and bowels.

Farnsteiner (*cf.* page 101) has found not only small quantities of linolic acid, but also traces of linolenic acid in lard.

Of the fat taken from different parts of the body, that of the head and ham is less consistent than that of the back and breast, while that of the flare has the greatest consistency.

Hehner and Mitchell § determined the amount of stearic acid in the fat from various parts of the same pig, and arrived at the conclusion that in the case of that particular animal, at least, the variations in consistency were due not so much to the presence of different proportions of that acid as to fluctuations in the amount of the liquid fatty acids, taken as oleic acid.

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* *Loc. cit.*, ii. p. 116.

† *Cf.* p. 134.

‡ *Die Ernahrung des Menschen*, p. 115.

§ *Analyst*, 1896, p. 326.

Assuming the fats to contain no other unsaturated fatty acid than oleic acid, and that the iodine absorption furnished a correct measure of the amount of that acid, they obtained the following approximate results:—

Fat of a Pig	Oleic Acid.	Stearic Acid.	Palmitic Acid.
from	Per cent.	Per cent.	Per cent.
Head, . . .	75	9	16
Ham, . . .	68·5	8·8	22·7
Breast, . . .	71	11·5	17·5
Flare, . . .	58·5	15	26·5
Back, . . .	75	8·8	16·2

The amounts of stearic acid contained in the saturated fatty acids calculated on this basis were:

Head.	Ham.	Breast.	Flare.	Back.
35	28	39·5	36·5	36

A summary of the physical and chemical constants of pigs' fat is given in the table on page 57, which also includes, for the purpose of comparison, some of the figures recorded for genuine lard, and the results obtained by Amthor and Zink, with a specimen of the fat of a wild boar.

HORSE FLESH.

Statistics.—In this country horse flesh has never been accepted as an article of human food like it has been on the Continent, and although it is probably eaten to a greater extent than is commonly supposed, popular prejudice on the subject compels the consumer to observe secrecy in his dealings with the vendor. Hence he loses the protection of having the meat under the surveillance of an inspector, as would be the case if it were sold as human food.

By the *Sale of Horse Flesh Act*, 1889, the vendor of horse flesh is compelled, under a penalty of £20 for non-compliance, to notify the nature of what he sells in conspicuous letters of at least three inches in length. For the purposes of the act 'horse flesh' includes the flesh of asses and mules.

According to Humbert,* over 20,000 horses were slaughtered in Paris in 1892 for human food, most of the flesh being manufac-

* *Journ. Pharm. Chim.*, 1895, 195–198.

CONSTANTS OF PIGS' FAT.

Description.	Specific Gravity.	Melting Point.		Solidifying Point.	Iodine Value.		Saponification Value, Mgrms. KOH.	Hehner Value.	Authority.
		Fat.	F. Acids.	F. Acids.	Fat.	F. Acids.			
	(At 100° C., Water at 15°=1)	°C.	°C.	°C.					
Mean results, 8 animals. Fat from back, . .	0·8607	33·8	40	..	60·58	61·90	..	..	Spaeth.
" kidney, .	0·8590	43·2	43·2	..	52·60	54·20	..	..	
" leaf, . .	0·8588	44·5	42·9	..	53·10	54·40	..	..	
Somersetshire pig, 6 months old. Fat from head, . .	..	..	34·8	..	..	67·7	..	..	Hehner and Mitchell, *Analyst*, 1896, 326–327.
" ham, . .	..	..	34·6	..	..	61·6	..	..	
" breast, .	..	..	36·8	..	..	64·2	..	..	
" flare . .	..	..	40·0	..	..	52·8	..	..	
" back, . .	..	..	35·6	..	..	67·9	..	..	
American pig. Fat from head, . .	..	29·5	..	..	68·79	..	..	..	Schweinitz and Emery, *Jour. Amer. Chem. Soc.*, 1896, 174–179.
" intestine, .	..	40·7	..	..	58·74	..	..	..	
" leaf, . .	..	..	..	..	56·85	..	..	..	
American pig. Fat from foot, . .	..	35·1	..	..	77·28	..	..	..	Wiley.
" head, . .	..	35·5	..	..	85·08	..	..	..	
" leaf, . .	..	44·5	..	..	52·55	..	..	..	
Mixed fat. (Lard), . . .	0·8610–0·8614	..	..	..	..	..	..	..	Gladding.
								93–95	Wiley.
American, ...	0·8595	..	..	40·2–41·9	47·7–58·6	..	..	..	Tennille.
" (regarded as highest limit), .	..	..	..	..	66·0	..	..	..	von Raumer.
	..	..	..	..	..	..	195·8–196·6	..	Valenta.
	..	36–45·5	..	..	49·9–63·8	..	..	..	Dieterich.
						Liquid F. Acids.			
German, . . .	..	..	..	..	..	94–96·2	..	..	von Raumer.
American, . . .	..	..	..	..	..	104	..	..	
Wild boar, . . .	At 15° C. 0·9424	40–44	39–40	32·5–33·5	76·6	81·2	195·1	..	Amthor and Zink.

tured into sausages, which were supposed to be sold with a declaration as to their nature.

Bremer* states that the number of horses slaughtered in the public abattoirs of Prussia were:—In 1891–92, 52,934; 1892–93, 52,543; 1893–94, 58,306; the number in Berlin alone amounting to nearly 10,000 per annum.

In Vienna, in 1892, 18,209 horses were killed, the numbers having steadily increased from 943 in 1854, when the first public slaughter-house was established. The price per pound of the flesh has also risen with the number of animals killed. In 1875 it cost 3d. per pound—more than twice what it fetched in 1856; and its price is said to be still on the increase.

Characteristics.—Horse flesh is coarser in texture and darker in colour than beef, and has a more distinctive and less pleasant odour. After standing for some time it develops a peculiar soapy feeling to the touch and a sickly smell, and its surface assumes a characteristic iridescent appearance. The muscular tissue of the horse, like that of the ox, darkens on treatment with alcoholic potassium hydroxide—a reaction which has been employed to distinguish horse flesh from pork.

It also develops a peculiar odour on treatment with formaldehyde (p. 134).

The average composition of horse flesh is shown in the table on p. 47, and the methods of detecting it in sausages are described at length on pp. 133–142.

The Fat.—Horse fat varies in colour from light yellow to deep orange, and has a consistency similar to that of butter. It contains on the average considerably more of the liquid fatty acids than do the animal fats described on the preceding pages.

In one case Hehner and Mitchell found a specimen of kidney fat to contain no stearic acid, but by crystallizing a large bulk of another specimen from ether, an insignificant deposit of crystals was obtained which closely resembled the characteristic forms of beef-stearin—a result which pointed to the presence of a trace of stearic acid.

Farnsteiner (p. 101) found 9·9 per cent. of linolic acid, but no linolenic acid, in a specimen of horse fat.

Amthor and Zink obtained an acetyl value of from 6·64 to 13·74 with different specimens of fat, by the original method of Benedikt and Ulzer, which, however, as Lewkowitsch† has pointed out, is liable to give erroneous results.

On the difference in the character of the fat extracted fromthe muscular tissue, Bremer has based a method of detecting horse flesh in the presence of pork.‡

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* *Forschungs Berichte*, 1897, p. 1.

† *Journ. Soc. Chem. Ind.*, 1897, p. 503.

‡ *Cf.* p. 141.

CONSTANTS OF HORSE FAT.

Description.	Specific Gravity.	Melting Point.		Solidification Point. F. Acids.	Iodine Value.		Saponification Value. Mgrms. KOH.	Hehner Value.	Authority.
		Fat.	F. Acids.		Fat.	F. Acids.			
	At 15° C.	°C.	°C.						
Fat from kidneys,	0·9320	39	36–37	30–30·5	81·09	83·88	198·7	95·4	Amthor and Zink.
,, neck, .	0·9330	34–35	32–33	32–33	74·84	74·41	199·5	95·42	
,, leaf, .	0·9319	36–37	31–32·5	31–32·5	81·6	83·37	197·8	94·78	
	At 17·5°.								
Fat from back, .	9159	...	52–53	45–43	79·9	81·4	...	...	R. Frühling, *Zeit. ang. Chem.*, 1896, 352–353.
,, heart, .	9167	...	40–44	34–32	77·4	78·3	...	...	
,, kidneys,	9212	...	53–54	48–47	82·6	84·0	...	...	
Mean results, .	9180	...	48·8	41·5	80·0	81·6	...	...	
Fat from kidneys,	...	18°	37·2	...	85·4	...	195·7	...	Hehner and Mitchell, *Analyst*, 1896, 328.
	15° 9189	41·8–43·2	37·5–39·5	37·3–37·7	86·1	83·9–87·1	195·1–196·8	96–97·8	Kalmann.
Intermuscular fat,	...	...	...	...	...	75·8 Liquid F. Acids. 108·1	...	...	Bremer, *Forsch. Ber.*, 1897, 1–8.

THE FLESH OF WILD ANIMALS AND BIRDS.

As was mentioned before (p. 48), the flesh of wild animals differs from that of the animals included in the preceding class in its closer texture and in containing much less fat. But this distinction is largely due to the different conditions under which the animals live.

The kind of food given to an animal has a considerable influence on the flavour of the flesh. Where fish has been the chief food the flesh acquires a fishy taste, and there is a marked difference in the flavour of a wild and tame duck.

The following are some of the principal average results of the analyses of the flesh of animals in the group. They are taken from the long table given by König*:—

	Per Cent.					Per Cent. Calculated on the Dry Substance.		
	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.	Nitrogenous Substances.	Fat.	Nitrogen.
Hare, . .	74·16	23·34	1·13	0·19	1·18	90·34	4·37	14·46
Rabbit, . .	66·85	21·47	9·76	0·75	1·17	64·77	29·74	10·84
Deer, . .	75·76	19·77	1·92	1·42	1·13	81·86	7·92	13·10
Hen (lean),	76·22	17·72	1·42	1·27	1·37	82·93	5·97	11·25
„ (fat) .	70·06	18·49	9·34	1·20	0·91	61·76	31·19	9·88
Duck (wild),	70·82	22·65	3·11	2·33	1·09	77·59	10·62	12·92
Goose (fat),	38·02	15·91	45·59	...	0·48	38·02	73·55	4·11
Pigeon, . .	75·10	22·14	1·00	0·76	1·00	89·58	4·17	14·23

Schlossberger and von Bibra obtained the subjoined results (see Table, page 61).

Bear's Flesh.—Strohmer† gives the following as the percentage composition of bear's flesh:—Water, 65·14; nitrogenous substances, 26·37; fat, 5·41; and ash, 1·44.

The Fat.—With one or two exceptions, the fat of the animals referred to in the preceding table has been but little examined, and in many cases the constants have been determined only with the fat of one individual.

The most complete research on this point is that of Amthor and Zink,‡ who have determined all the usual constants of the fat of a large number of animals and birds. They found that as a rule a high specific gravity of the fat was accompanied by a high melting point and low iodine value. The iodine value of the fats and fatty acids decreased on keeping, whilst, on the other hand, in the case of many fats, such as that of the stag, dog, and wild boar, the acid value increased. The iodine value and, generally, the acetyl value* were lower in the fat of domestic animals than in that of the corresponding wild animals. A similar difference was observed in the case of the birds, the fat of the domestic goose, hen, and duck being lard-like, while that of the related wild birds was oily. In four cases the fats had marked drying properties, three of them becoming quite solid in the course of seven or eight to twelve days when spread in a thin layer on a glass plate. This property was possessed by the fat of the hare, wild rabbit, wild boar, and, in a lesser degree, by that of the blackcock.

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* *Loc. cit.*, ii. p. 118.

† *Die Ernahrung des Menschen.*

‡ Abst. *Analyst*, 1897, p. 75.

Flesh of	Water.	Nitrogenous Substances.		
		Albumin.	Flesh Fibre.	Gelatin-yielding.
Roe, ...	74·63	1·94	16·81	0·50
Do., ...	78·30	2·30	18·00	...
Hen, ...	77·30	3·00	16·50	1·20
Wild Duck, . .	71·76	2·68	17·68	1·23
Pigeon, . . .	76·00	4·50	17·00	1·50

The fat of the wild boar differed from common lard in having a higher specific gravity, iodine value, and acetyl number, and especially in the above-mentioned drying property.

Fox fat differed from that of the cat and dog in its higher iodine value and specific gravity, but more particularly in its high acetyl value.* The principal differences in the fats of the common and wild cats were the higher Reichert and acetyl numbers and the considerably higher acid values of the latter. Pole-cat fat was quite liquid and had somewhat lower constants than the fat of the marten. The fat of the dog and cat were very similar in appearance to lard, which they also resembled in analytical constants, with the exception of the acetyl value, which was higher.

Of the different bird-fats examined, that of the blackcock was noticeable for its drying properties and high iodine value. When spread on glass it soon set to a varnish, which, however, still remained sticky. After fourteen days drying was still proceeding. After ninety-five days the iodine value had fallen to 29·7.

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* But *cf.* note, p. 58 (†).

The chemical and physical constants of the fat of some of the principal animals of this group, which are used for food, are shown in the subjoined table (page 63).

The 'Ripening' of Game.—When game is allowed to hang for some time in a whole condition the flesh undergoes an alteration which Eber considered was of a purely chemical nature, although he termed it 'acid fermentation.' The reaction of the flesh becomes strongly acid, the muscular tissue becomes more tender, and after some time traces of hydrogen sulphide are liberated. Eber found that the production of the characteristic flavours of game stood in direct proportion to the amount of hydrogen sulphide or mercaptans set free; but in his opinion the flavour (*haut goût*) was in no way due to the formation of putrefactive compounds. A similar ripening process can be brought about in the flesh of other animals besides game, and indeed is necessary in the case of old cattle, or of bulls.

As apparently Eber did not make a bacteriological examination of the flesh before and after 'ripening,' his view appears unlikely to be correct, and it is far more probable that the change is brought about by certain species of bacteria.

The 'Heating' of Game.—When game is packed too closely and subjected to too high a temperature, an alteration rapidly takes place, which is probably, in the main, a chemical change, brought about by bacterial products rather than by the bacteria themselves. Within an hour or two the skin becomes green, the hair loose and easily removed, and the muscle soft and flabby, while bubbles of gas may often be observed within the tissue. The reaction of the flesh is very acid, and considerable quantities of hydrogen sulphide are evolved, so that the flesh has a disagreeable odour.

Eber* succeeded in imitating the green coloration and acid formation by injecting milk of potassium sulphide (0·5 per cent.). The origin, however, of the excess of sulphur compounds in the natural 'heating' is doubtful, though it is probably due to the rapid permeation of bacterial products from the large intestine.

That the alteration is not an ordinary putrefactive process is shown by the facts that no ammonia is produced, and that the change is not progressive, *i.e.* on removing the causes (the close packing and high temperature) the muscle retains for a considerable period its consistency and original structure.

This condition of the flesh may also occur in the flesh of other

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* *Zeit. f. Fleisch u. Milch Hyg.*, 1897, vii. p. 208.

CONSTANTS OF THE FAT OF WILD ANIMALS AND BIRDS.

Fat from	Sp. gr.	Melting Point ° C.		Solidification Point F. Acids. ° C.	Iodine Value.		Saponification Value = Mgrms. KOH.	Hehner Value.	Reichert Value.	Authority.
		Fat.	F. Acids.		Fat.	F. Acids.				
	15° C.									
Stag,	0·9670	51–52	50–52	46–48	25·7	23·6	199·9	..	1·66	Amthor and Zink.*
Fallow Deer,	0·9615	52–53	50–53	47–48	26·4	28·2	195·6	..	1·70	
Roebuck,	0·9659	52–54	62–64	49–50	32·1	27·9	203·3	..	0·99	
Hare,	0·9349	35–40	44–47	36–40	102·2	93·3	200·9	95·2	1·59	
Rabbit (tame),	0·9342	40–42	44–46	37–39	69·6	64·4	202·6	..	2·80	Amthor and Zink.*
,, (wild),	0·9393	35–38	39–41	35–36	99·8	101·1	199·3	..	0·70	
,,	0·861 (at 100°)	44–46	48–50	39–41	..	..	..	95·47	2·64	L. Drumel.†
Goose (from 4 parts of same bird),	0·9228 to 0·9300	27·5 to 31·7	36·6 to 40.2	..	58·7 to 66·4	..	191·2 to 193·0	94·5 to 95·3	0·2 to 0·3	J. Rozsenyi.‡
,, ,,	0·9274	32–34	38–40	31–32	67·6	65·3	193·1	..	0·98	Amthor and Zink.*
,, (wild),	..	..	34–40	33–34	99·6	..	..	..	..	
,, ,, §	0·9158	..	36–38	32	67·0	65·1	196·0	..	..	
Duck,	..	36–39	..	..	58·5	..	..	..	..	,,
,, (Wild),	..	..	36–40	30–31	84·6	..	198·5	..	1·30	,,
Turkey,	0·9241	33–40	38–40	32–34	66·7	64·6	193·5	..	1·00	,,
Blackcock,	0·9220	..	38–39	31–32	81·15	70·7	200·5	..	..	,,
,,	0·9296	..	30–33	25–28	121·1	120·0	201·6	..	2·10	,,
Pigeon,	..	..	38–39	33–34	82·3	..	..	..	..	

* *Zeit. anal. Chem.*, 1897, p. 1. † *Bull. de l'Ass. belge*, 1896, ix. 323. ‡ *Analyst*, 1896, p. 235. § Two years in captivity.

animals from which the skin has not been removed in the summer. Eber has frequently observed it in the case of horses.

According to Fischoeder,* 'heated' flesh is not allowed to be sold in Germany, although no ill results have been traced with certainty to its use.

THE FLESH OF VERTEBRATE FISH.

The muscular tissue of fish is generally white, and is less consistent than that of land animals. The difference in colour is due to the flesh and the blood retained by it containing so much less hæmoglobin. In certain fish, however, such as the salmon, the flesh has a delicate pink tint which Krukenberg attributes to the presence of a colouring matter of the nature of a lipochrome. The flesh of fish usually contains a high percentage of water, often as much as from 80 to 85 per cent.; the amount of mineral matter is also usually high, sometimes amounting to nearly 2 per cent.

In certain fish special constituents have been found, as, for instance, isokreatinine in the haddock (*cf.* p. 10).

Fish undergo decomposition with extreme readiness, and in many cases the products of decomposition are deadly poisons to the human system. Occasionally, too, the living fish elaborate poisonous substances. These may be of the nature of leucomaines or albuminous toxines, and are probably in some instances due to the condition of the water in which the fish have been living.†

The age of carp may be approximately estimated in the following manner:—A scale from the side of the fish is cleansed with alcohol and held to the light. If only a bright central spot is visible the carp is only one year old. If one ring surrounds the central point the fish is two years old; if two rings, three years old, and so on. Experiments have shown that the number of rings regularly increases with the age.‡

The Fat of Fish.—It is mainly to the presence of certain constituents in the fat that the characteristic flavour of different kinds of fish is due. In some fish the liver is the chief source of the fat, as, for instance, in the case of the cod and shark, while the rest of the body contains only a small proportion. In other fish the oil is distributed throughout the body, and is not specially abundant in the liver.

The fat is of an oily nature, and as a rule contains a smaller proportion of the compounds of the solid fatty acids than does the

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* *Leitfaden der prak. Fleischbeschau*, 1897, p. 199.

† *Cf.* pp. 219-222.

‡ *Zeit. Fleisch u. Milch Hyg.*, 1897, p. 244.

The following analytical figures selected from König's table * show the percentage composition of some of the better-known kinds of fish.

Fish.	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.	Calculated on the Dry Substance.		
						Nitrogenous Substances.	Fat.	Nitrogen.
A. Fish Rich in Fat—								
Salmon,	64·29	21·60	12·72	..	1·39	60·49	35·62	9·69
Eel (sea),	71·45	18·46	9·09	..	1·00	64·76	31·69	10·36
Herring,	74·64	14·55	9·03	..	1·78	56·42	35·35	9·03
Mackerel,	71·20	19·36	8·08	..	1·36	67·22	28·06	10·75
Halibut,	75·24	18·53	5·16	..	1·06	76·54	19·11	12·23
B. Fish Poor in Fat—								
Pike,	79·63	18·42	0·53	0·46	0·96	90·59	2·42	14·50
Carp,	76·97	21·86	1·09	..	1·33	94·22	4·73	15·19
Flounder,	84·00	14·03	0·69	..	1·28	87·66	4·38	14·02

Some of the more recent results of A. Bolland † are as follows:—

Fish.	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.	Dry Substance.			
						Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.
River Eel,	59·3	13·05	25·69	0·70	0·76	32·46	63·90	1·74	1·90
Herring (fresh),	76·0	17·23	4·80	0·46	1·51	71·80	20·00	1·90	6·30
Mackerel,	67·6	15·67	15·04	0·28	1·41	48·37	46·41	0·88	4·34
Perch,	82·6	14·90	0·55	0·98	0·97	85·63	3·16	5·61	5·60
Salmon,	61·4	17·45	20·00	0·08	0·87	45·72	51·82	0·20	2·26

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* *Loc. cit.*, ii. p. 121.

† *Comptes Rend.*, 1898, cxxvi. p. 1728.

CONSTANTS OF CERTAIN FISH OILS.

Fat from	Specific Gravity at 15° C.	Melting Point, ° C.		Solidification Point, F. Acids, ° C.	Iodine Value.		Saponification Value = Mgrms. KOH.	Hehner Value.	Acid Value.	Authority.
		Fat.	F. Acids.		Fat.	F. Acids.				
Sardine, . .	0·933	...	...	...	193·2 191·7	...	190·9	94·5	4·6 to 21·7	Fahrion.* †
Cod (liver), .	0·922 to 0·927 (K)	...	48 to 52 (K)	13·3 to 24·3 (L)	123 to 141 (K)	...	171 to 189 (K)	95·3 (L)	0·62 to 28·67 (K)	Kremel.* Lewkowitsch.*
,, (body), .	0·9227	−3	21·2	16·5	137·5	...	173·6	...	7·6	Mitchell. ‡
Haddock (liver), .	0·9298	...	...	...	154·2	...	188·8	93·3	...	Lewkowitsch. §
Skate (liver), .	0·9307	...	...	...	157·3	...	185·4	94·7	...	Lewkowitsch. §
Shark (Japanese),	0·9177	...	...	...	136·0	...	163·5	...	0·88	H. Bull. ‖
,, (Arctic) (liver), . .	0·9163	...	...	...	114·6	...	161·0 ¶	86·9	...	Lewkowitsch. §
,, . .	...	...	...	...	138·6	...	...	97·26	8·2	Fahrion.†
,, . .	0·9189	−4	21·8	17·4	133·1	124·3	...	...	11·3	Mitchell.‡
Herring (Japanese),	0·9215	...	...	...	131·0	...	170·9	...	1·8	H. Bull. ‖
,, (English),	0·9891	...	...	...	132·7	...	184·8	...	40·2	

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* Benedikt and Lewkowitsch, *Oils, Fats, and Waxes*, pp. 388, 391, 392.
† *Analyst*, 1899, pp. 136, 186.
‡ Not previously published.
§ *Loc. cit.*, p. 401.
‖ *Journ. Soc. Chem. Ind.*, 1900, p. 177.
¶ Unsaponifiable matter, 10·20 per cent.

fat of land animals.[*] It is mainly composed of glycerides of various unsaturated acids. The liver-oils usually contain certain bile products, which give rise to characteristic colour reactions with acids and alkalies. A considerable proportion of unsaponifiable matter, principally cholesterin, is also a usual constituent.

With the exception of cod-liver oil, the fish fats have been but little studied, and our knowledge of their physical and chemical characteristics is therefore limited.

Hehner and Mitchell[†] have found that from marine animal oils —cod-liver, cod, shark, whale—compounds can be obtained which have many similarities with those prepared in the same way from linseed oil, though they have not the same drying capacity.

For a full description of the nature of cod-liver oil and the methods of detecting its adulteration, reference must be made to works dealing specially with the examination of fats and oils.

The previous table (page 66) gives some of the constants which have been obtained with fats of this class.

THE FLESH OF INVERTEBRATE ANIMALS.

The flesh of invertebrate animals does not present any marked difference in structure to that of other animals. In chemical composition it differs, as a rule, from that of vertebrate animals in containing more water, nitrogen-free extractives and ash, and less fat.

According to Bolland, the acidity of crustacea, calculated on the original substance, varies from 0·038 to 0·258 per cent.

König[‡] gives the following percentage composition of some of the members of this group:—

	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.	Calculated on the Dry Substance.		
						Nitrogenous Substances.	Fat.	Nitrogen.
Oyster, Flesh, .	80·5	9·04	2·04	6·44	1·96	46·41	10·47	7·43
" Liquid, .	95·76	1·42	0·03	0·70	2·09	33·49	0·71	5·36
" Flesh and Liquid,	87·30	5·95	1·15	3·57	2·03	46·85	9·06	7·50
Mussel, . .	84·16	8·69	1·12	4·12	1·91	54·86	7·07	8·78
Lobster, . .	81·84	14·49	1·84	0·12	1·71	79·80	10·13	12·77
Crab, . . .	79·97	15·80	1·54	0·75	1·94	78·87	7·69	12·62

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[*] Excluding the liquid fats extracted from the feet, such as neatsfoot oil, etc.

[†] *Analyst*, 1898, p. 317.

[‡] *Loc. cit.*, ii. p. 130.

The following representative analyses are taken from A. Bolland's table * :—

	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.	Dry Substance.			
						Nitrogenous Substances.	Fat.	N.-free Extractives.	Ash.
Crab, .	76·5	15·89	0·87	5·75	0·99	67·60	3·69	24·50	4·21
Cockle, .	92·0	4·16	0·29	2·32	1·23	52·00	3·67	29·00	15·33
Oyster, .	80·5	8·70	1·43	7·33	2·04	44·60	7·32	37·61	10·47
Mussel, .	82·2	11·25	1·21	4·04	1·30	63·20	6·82	22·68	7·30
Burgundy Snail, .	79·3	16·10	1·08	1·97	1·55	77·88	5·20	9·52	7·50
Weinberg Snail, .	80·5	16·34	1·38	0·45	1·33	83·78	7·10	2·32	6·80

A. Chatin and A. Muntz † have determined the amount of phosphorus in different varieties of oysters. In 100 parts of the dry flesh they found 1·836 parts in French oysters, and 2·082 parts in Portuguese oysters. A Portuguese oyster of medium size contained 0·032 gramme, and a similar French oyster 0·02 gramme of phosphorus, which was present in combination with organic substances. Iron was also present.

Green Oysters.—From the recent researches of Boyce and Herdman ‡ there appear to be several kinds of greenness in oysters. In some varieties, such as the Marennes oysters and those from rivers on the Essex coast, the green colour is a normal and healthy condition, and is to be attributed to the presence of a pigment termed 'marennin' and not to an excess of copper. This confirms Ray Lankester's conclusions, who found that the copper in green Marennes oysters was not greater than that normally present in the hæmocyanin of the blood of colourless oysters. The average amount of copper found by Kohn in his analyses for Herdman and Boyce in different varieties (including those of Marennes) was 0·006 grain.

In some kinds of greenness, however, there is undoubtedly a larger proportion of copper than this normal quantity. In certain American kinds, and in oysters from Falmouth, Herdman and Boyce found patches of green on the mantle and in the heart, which were derived from an excess of copper in the blood leucocytes. This they attributed to a diseased condition of the blood which they term 'green leucocytis.' The quantity of copper in the green leucocytes varied considerably, some giving a marked brown coloration with potassium ferrocyanide, while others gave only a faint reaction. Occasionally iron was also found. In the case of the Falmouth oysters, part of the excess of copper was mechanically attached to the body, probably as basic carbonate. Attempts to produce the greenness artificially by feeding oysters with dilute solutions of copper or iron salts were unsuccessful.

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* *Comptes Rend.*, 1898, cxxvi. p. 1728.

† *Compt. Rend.*, 1895, p. 1095.

‡ *Proc. Roy. Soc.*, 1897, lxii. p. 30, and 1899, lxiv. p. 239.

CHAPTER IV.

THE EXAMINATION OF FLESH.

The absolute value of flesh as food, apart from the relative value which depends on the nature of the animal from which it was taken or its position in the animal, is largely based (1) on its physical external characteristics, such as colour, consistency, etc.; (2) on the proportion of fat; and (3) on its taste and odour.

COLOUR.

The normal colour of sound flesh varies with its origin, ranging from white, as in the case of many fish, to dark purple-red, as in horse flesh. The colour being largely dependent on the amount of hæmoglobin in the flesh, an approximate ratio may often be observed between the colour and the amount of iron (chiefly derived from the hæmoglobin) in the ash, left on calcining the flesh.

Abnormal Colorations of Flesh.

Melanosis.—Morot[*] describes a case in which the flesh of a calf three months old was found to contain numberless small black specks distributed throughout the body. Sometimes this is only local, and not, as in this instance, general. The black pigment is probably a derivative of hæmoglobin. In Germany such flesh is sold at a low price by the Freibank (p. 215).

White Flesh.—This is found normally in certain full-grown foreign animals. Occasionally it may happen that the flesh of a cow or ox does not acquire the usual amount of hæmoglobin and has the appearance of veal. Faucon[†] records a case of the kind in which the muscular tissue of a cow four years old had all the appearance of a calf's flesh, differing only in the larger size of the muscular fibres and in its greater dryness. White flesh is found in certain diseases, such as the anæmia of dropsy, and is probably caused by an insufficient oxidation of the blood.

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[*] *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 501.

[†] *Ibid.*, 1898, p. 34.

Yellow Colour.—This is sometimes produced by the food given to the animal. In disease it is due to biliary compounds being absorbed and retained by the flesh.

Brown Flesh (‘*Xantosis*’).—An instance of this is described by Goltz, in which flesh had a brown coloration due to the presence of granules of a yellowish-brown pigment between the fibres.

Dark Purple.—This may indicate that an animal has suffered from acute fever, and it is met with in animals that have died of rinderpest or tuberculosis. It may also be due to the animal having died a natural death, so that the blood has been retained in the body, or to insufficient bleeding after death, due to weak action of the heart.

Dark Reddish-Brown.—This is due to imperfect oxidation of the blood. It is observed in the case of animals which have been drowned or suffocated in smoke (CO_2 poisoning). There is also often considerable discoloration in the flesh of animals which have been hunted or over-driven.

Scarlet.—This is but seldom met with. It indicates carbon monoxide poisoning, and is sometimes a result of arsenic poisoning (Walley).

Diffused Redness.—This is due to the diffusion of hæmoglobin, and is often seen in meat which has been frozen. It is also found in cases of blood-poisoning.

Iridescence.—This is a normal characteristic of horse flesh. In other animals it occurs as the result of certain diseases of the blood.

Green or Violet Hue.—This may be due to the commencement of putrefaction, or to the diffusion of vegetable colouring matter through the membrane of the stomach after death (Walley). (See also Green Oysters, p. 68.)

Colorations due to the Direct Action of Bacteria.

These are referred to under the heading of ‘Chromogenic Bacteria on Flesh,’ p. 270.

Artificial Coloration of Flesh.

Kellermann* has recently detected saffron in a sample of so-called smoked pork. The connective tissue was very yellow, while the muscle had the colour of ordinary flesh. It had not the usual smell of smoked flesh. On treatment with alcohol an intensely yellow solution was obtained, which, on evaporation, left a residue giving a violet coloration with sulphuric acid.

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* *Zeit. f. Untersuch. Nahr. Genussm.*, 1898, p. 247.

An account of the various substances used to colour sausages and meat preparations, and the means of detecting them, is given in a subsequent chapter.*

The 'Flesh Juice' ('Roseline') of Adamczyk, Berlin, consists of red carmine lake in ammoniacal water.

UNSOUND FLESH.

In this country the inspectors condemn the flesh of animals which have died from natural causes, or as the result of an accident, as well as of those which have suffered from certain diseases, such as pleuro-pneumonia, puerperal fever, etc. Meat which is tainted with chemical substances, such as phenol, or in a state of putrefaction, or infected with animal parasites, such as liver flukes or hydatids, is also obviously rejected.

To distinguish the meat of diseased animals cannot, as yet, be done with any certainty by chemical methods, and it requires considerable practice to form a correct judgment of the character of flesh by its appearance. The bacteriological methods of examination are described in Chap. XIII.

General Characteristics of Sound Flesh.—According to Letheby,† sound fresh meat has the following general characteristics:—1. The colour is neither very pale nor dark purple. 2. It has a marbled appearance, due to the presence of small veins of fat distributed throughout the muscle. 3. It is firm and elastic to the touch, and not sodden or flabby, and scarcely moistens the finger. 4. It is free from objectionable odour. 5. It does not become wet on standing for a day or so, but, on the contrary, gets drier. 6. It does not lose more than 70 to 74 per cent. in weight when dried at 100° C., whereas bad meat often loses more than 80 per cent. 7. It does not shrink much in cooking.

Chemical Tests for Unsound Flesh.

Apart from alterations in the appearance of the parts locally affected with the disease, it is probable that chemical alterations also occur in the flesh through the products of the bacterial action permeating the muscular tissue of the living animal. So far the only attempt to differentiate diseased from healthy flesh on these lines is that of Professor Eber, who was still working on the subject at the time of his death.[*]

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* *Cf.* p. 142.

† *On Foods*, p. 210.

His test is based on the fact that in the case of diseased animals, especially those suffering from tuberculosis, there is often a considerable increase in the amount of readily decomposable sulphur compounds in the flesh, which can be approximately measured by noting the relative amount of hydrogen sulphide (or mercaptans) evolved on treating the flesh with dilute acid.

Although hydrogen sulphide is often one of the products of putrefactive decomposition, its presence does not necessarily denote putrefaction. Thus traces of it are normally present in the free state in all old flesh, of which the reaction is still acid, and considerable quantities are evolved during the 'heating' ('*verhitzein*') of game.[†] That the volatile sulphur products of pathogenic bacteria may be rapidly diffused throughout the body is shown by the fact that the carcasses of pigs killed on account of swine erysipelas turn green after the lapse of a very short time (thirty minutes), and emit an unpleasant odour; and, as the flesh still has an acid reaction, putrefaction is here out of the question.

The muscles, kidneys, and lymph-glands of healthy recently-killed animals evolve hydrogen sulphide when heated in a test-tube on the water-bath, the gas being probably derived from the decomposition of proteid matter; but Eber could not notice any difference in the quantities thus yielded by healthy compared with diseased flesh.

The more limited decomposition brought about by the action of dilute sulphuric acid gave more promising results, which Eber was intending to confirm and extend.

Eber's Hydrogen Sulphide Test.[‡]—From 10 to 25 grammes of the finely divided substance are mixed with 50 grammes of dilute sulphuric acid, (1 : 10) in an Erlenmeyer flask, in the neck of which is fixed, by means of a loose plug of cotton-wool, a strip of filter-paper previously soaked in a 10 per cent. solution of lead nitrate. The flask is kept in the dark for twenty-four hours in a spot to which there is free access of air, without a draught, at a temperature which may vary from 12° to 18° C., without influencing the results. At the end of the time the strip is gummed in a book, and after the lapse of thirty minutes compared with a standard scale of strips.

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[*] W. Eber, born 1863, died June 1898.

[†] *Cf.* p. 62.

[‡] *Zeit. Fleisch u. Milch Hyg.*, 1897, vii. pp. 207-211, 227-231; and viii. pp. 41-46.

The colour of the strip will vary from faint brown (rarely yellow) to black, the lower part being the most intense.

The colour scale,* of which the relative shades are shown in the frontispiece, is an arbitrary one, and the absolute value of each colour was not definitely determined by Eber. Preliminary experiments, with a solution of potassium sulphide yielding an accurately determined amount of hydrogen sulphide, indicated that the lowest colour in the scale No. 1 corresponded to 0·002 milligramme of hydrogen sulphide, while No. 8 (black) was first attained with 0·01 milligramme.

By this method Eber found that the mean results obtained with the flesh of different kinds of animals were, as a rule, somewhat higher in cases of tuberculosis (viz., 4·6 as against 3·5), whilst the tests with the ileo-lumbar glands often showed a considerable difference, as is seen in the following figures:—

i. Healthy, . .	130 samples.	100 negative.	mean 0·2
ii. Local tuberculosis,	44 „	8 „	„ 1·6
iii. General tuberculosis,	36 „	3 „	„ 2·7

The results given by the kidneys were invariably high (6 to 7), and no difference could be found between those of healthy and of diseased animals.

As these figures were obtained without the precaution of excluding light, which, as was subsequently found, tends to lower the value, it was Eber's intention to repeat the tests.

Much more work is required on the subject before this test can be regarded as having passed the experimental stage, but it indicates a starting-point for fresh departures, and has possible applications in other directions. Thus it may be found to serve as a measure of the *haut goût* of game,† and may be employed to elucidate the progress of the changes which occur in the putrefaction of flesh.

The Ptomaines formed during Putrefaction.—The methods of isolating the basic alkaloidal products formed during the decomposition of flesh by saprophytic bacteria, and the characteristics of individual ptomaines, are described in Chap. XIV., p. 298.

The Reaction towards Litmus Paper.—A slice is cut from the meat and pressed against a piece of moistened litmus paper. After ten minutes the paper is removed and compared, on a white surface, with a piece of the original litmus paper similarly moistened.

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* The original colour scale may be obtained from R. Schötz, Luisenstrasse 36, Berlin, N.W.

† *Cf.* p. 62.

As a general rule, flesh becomes acid soon after death, and continues so until sufficient ammonia is produced during the progress of putrefaction to render it alkaline.*

According to Augst,† in cases of acute pneumonia, or other diseases causing shortness of breath, the flesh only assumes the normal acid reaction some twenty-four hours after death, and is alkaline until then.

Hartenstein ‡ records a similar phenomenon in the case of a cow which had been killed after suffering for five days from colic. The flesh had a marked alkaline reaction four hours after death, and it was not until the next day that it became acid.

Occasionally the result of the litmus test is a colour intermediate between the red and blue. This 'amphoteric' reaction occurs most frequently in the muscles of animals which have not been cut up, such as birds and fish.

The alkaline reaction is also obtained with certain organs of the body in a fresh condition, as, for example, the spleen. Pickled flesh and smoked ham are also, as a rule, strongly alkaline.

Eber's Test for Putrefaction.—To detect incipient putrefaction, Eber § recommended the use of a reagent, composed of hydrochloric acid, 1 part; alcohol, 3 parts; and ether, 1 part. A glass rod is moistened with this and brought near the meat, from which, if putrefaction has commenced, fumes of ammonium chloride are often produced. In order to avoid the chance of error, due to the fuming of the hydrochloric acid itself, a few c.c. of the reagent are introduced into a stoppered cylinder, which is shaken so as to moisten its sides, and a fragment of the meat introduced on the end of a wire.

The intensity of the cloud of ammonium chloride is not proportional to the odour of the flesh, for in some instances putrefaction may proceed without the evolution of malodorous substances, and in others these may only become evident on boiling the flesh. This is noticeable in the case of salt fish, in which there may possibly be a combination between the volatile bad-smelling compounds and the salt.

Sometimes Eber's reagent gives negative results when putrefaction has commenced, owing to the formation of acid substances, instead of or in excess of the ammonia. This is especially the case with liver and with game.

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* *Cf.* p. 19.

† *Deutsch. Tierärzt. Wochensch.*, 1897, p. 37.

‡ *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 68.

§ *Arch. Wissensch. u. prak. Thierheilk.*, 1893, xviii., and 1894, xix. p. 81.

Eber classified the results of this test in the following scheme :—

I. No cloud of NH_4Cl (absence of ordinary (alkaline) putrefaction).

$$\textit{Reaction of flesh,}\left\{\begin{array}{l}\text{Acid,}\\ \text{Amphoteric,}\\ \text{Alkaline,}\end{array}\right\}\begin{array}{l}(a)\text{ with bad odour,}\\ (b)\text{ without ,,}\end{array}\left\{\begin{array}{l}\text{i. with } H_2S.\\ \text{ii. without } H_2S.\end{array}\right.$$

II. Cloud (Putrefaction proper).

$$\textit{Reaction of flesh,}\left\{\begin{array}{l}\text{Acid,}\\ \text{Amphoteric,}\\ \text{Alkaline,}\end{array}\right\}\begin{array}{l}(a)\text{ with bad odour,}\\ (b)\text{ without ,,}\end{array}\left\{\begin{array}{l}\text{i. with } H_2S.\\ \text{ii. without } H_2S.\end{array}\right.$$

TREATMENT OF MEAT WITH ANTISEPTICS.

It is no uncommon practice for butchers to treat meat which has been kept too long with various chemical solutions in order to retard or disguise the commencement of decomposition.

Of these reagents the least objectionable is a dilute solution of **potassium permanganate**, which removes the odour from meat slightly tainted on the surface, but is incapable of disguising deep-seated decomposition.

Many of the preservative solutions contain **calcium** or **sodium sulphites** or **bisulphites** as a chief constituent. The salts of **sulphurous acid** have not only an antiseptic action, but possess the property of restoring the colour of flesh which has become brown or grey through exposure to the air.

The action of **sulphurous acid** on the meat fibres, and the methods of detecting it, are referred to on p. 121.

Borax and **boric acid** are also frequently used for this purpose. L. Baillet * records the results of experiments in which joints of mutton were steeped for a month in a solution of borax. The meat had nearly its normal red aspect, but the borax had penetrated a long way into the flesh, and could readily be detected by turmeric paper (see also p. 119).

According to de Cyon of St Petersburg, meat treated with a solution of boric acid acquires a disagreeable appearance and flavour.

The use of **salicylic acid** or **formaldehyde**, which are said to be employed for 'doctoring' meat, cannot be very general on account of their other effects (pp. 122–123).

As meat which has thus been treated is often in a state of arrested decomposition, the following tests will often be serviceable in detecting the fraud.

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* *Traité de l'Inspection des Viandes*, p. 523.

1. General appearance and physical characteristics, such as excess of moisture and want of firmness; meat which has been kept too long readily tears, and does not cut evenly.
2. The appearance and acid value of the fat (*cf.* p. 95).
3. The reaction of the meat-juice towards litmus and turmeric paper (p. 74).
4. The microscopical appearance of the fibres (p. 117).
5. The chemical identification of definite preservatives (pp. 118–124).

'BLOWN' MEAT.

With the object of imparting a better appearance, meat is sometimes 'blown' by butchers. This process consists in inflating the loose cellular tissue by means of bellows, or usually with the breath through a blow-pipe, and then blowing melted fat over the parts.

In Germany this disgusting practice is common in the case of calves and sheep, but is less frequently met with in beef.[*] In this country, according to O. Andrews, veal and lamb are occasionally 'blown.'

The blown-out parts appear larger, and have a shiny surface, while the muscle feels spongy and crackles under the pressure of the finger. Small air-bubbles may frequently be observed within the tissue. Lungs which have thus been 'blown' have rounded edges, and contain isolated air-bubbles.

CONSISTENCY OF FLESH.

The consistency of meat is a valuable criterion of its soundness. Good meat is firm to the touch, while unsound flesh is frequently flabby and exudes moisture. Coarse-grained meat, which cannot be cut evenly and regularly, is inferior to fine-grained.

The nature of 'grain' depends (1) On the age of the animal, the fibres being finer in the case of young animals; (2) On the race of animals and nature of their feeding; and (3) On the sex of the animal—the cow, for example, having flesh of finer fibre than the bull.

Determination of the Degree of Toughness.—Lehmann[†] has devised an ingenious apparatus for determining the degree of toughness of different food products. This consists of a balance with arms of different lengths, the shorter being constructed on the principle of a pair of scissors with one of the blades fixed. The weights are placed in the pan of the longer arm, and the force required to cut through a layer of the substance 1 cm. thick is expressed in grammes.

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[*] *Fischoeder.*

[†] *Zeit. Fleish u. Milch Hyg.*, 1898, viii. pp. 32-33.

With this apparatus Lehmann found that the skin muscle of beef is two and a half times as tough as the fillet, owing to the greater proportion of the white (collagenous) fibres of connective tissue in the former, and of elastic fibres in the latter. Collagenous fibres (sinews) required 1040 grammes, whilst elastic tissue required only 580 grammes for complete division.

Hence, flesh which contains much collagenous tissue becomes more tender on boiling, whereas that containing but little remains practically the same. For instance:—

	Raw, grammes.	Boiled, grammes.
Fillet of beef, ...	83·4	84·0
Skin muscle of beef (*Hautmuskel*),	236·4	88·8

The following values were also obtained with different parts of the body in the raw and cooked state:—

	Raw, grammes.	Boiled, grammes.
Heart, ...	104	88
Tongue (Hyoglossus), . . .	...	64
Liver, ox, ...	42	8
,, calf, ...	35	6·6
Kidneys, ...	40	24
Brain, ...	7·0	2·4

It is interesting to note that game, on hanging, loses in a few days 25 per cent. of its toughness.

THE ODOUR OF FLESH.

This is best observed on boiling fragments of the flesh with water, and in some cases by mixing the flesh with dilute sulphuric acid, distilling about a fourth part of the liquid, and noting the smell of the distillate. It may be:—

(i.) The normal odour characteristic of each kind of animal.
(ii.) The characteristic odour intensified to a very unpleasant extent, in the case of the flesh of uncastrated male animals. This is more marked with the flesh of the he-goat and boar than with that of the ram and bull.
(iii.) An abnormal odour, due to the substances (fish, for example) eaten by the animal.
(iv.) An odour due to chemical alteration or decomposition, as, for instance, that of the volatile products formed during the 'heating' of game, or the putrefaction of flesh.
(v.) An odour of foreign substances—phenol, chloride of lime, etc.

ANALYTICAL METHODS.

Determination of Water.—From 5 to 10 grammes of the finely divided flesh are dried in an air bath maintained at 105°-110° C. until the weight is constant.

If the substance is readily oxidizable it must be dried *in vacuo*, or approximate results may be rapidly obtained by the following method, used by C. Parsons * for sensitive organic bodies, in which the action of atmospheric oxygen is excluded during the drying:—A neutral mineral oil with a high flash test and boiling point is heated at 250° F. until its weight becomes constant. A basin containing some of the oil is weighed, heated at 240° F. for some minutes, a few thin pieces of the meat (previously weighed) added, and the heating continued until all effervescence ceases. The loss in weight of the basin containing the oil and the meat gives the amount of water expelled.

Abnormal Moisture.—Walley † gives a summary of the various causes leading to an excess of water in flesh. These are (1) Albuminous effusion, as in 'turnip braxy' in sheep (*cf.* p. 53), due to enzymic action in the cells of the living animal; (2) Effusion of serum or *hydræmia*, as in the 'water-braxy' of sheep. This is also found in diseases with inflammatory symptoms, such as erysipelas, and may originate from irritation set up by parasites (cysticerci). Effusion of serum occurs in meat which has been thawed after freezing. (3) Lymph effusion, either local or diffused, arising from inflammation or other disease. (4) Effusion of blood, either local or general, caused by violence or disease. (5) Effusion of urine, which is necessarily local.

Determination of Mineral Matter (Ash).—The residue left from the determination of water is ignited in a covered platinum dish, preferably in a muffle-furnace.

Iron, Calcium, and Magnesium.—The ash is dissolved in dilute hydrochloric acid, and the iron precipitated with ammonium acetate, the calcium with ammonium oxalate, and the magnesium with sodium phosphate.

Sodium and Potassium.—Warden and Bose ‡ determine these metals in the following manner:—The soluble ash is dissolved in water, barium chloride, ammonium chloride, and ammonia added, and the liquid heated and filtered. The filtrate is mixed with ammonium carbonate and ammonium oxalate, heated and filtered. The filtrate and washings are evaporated to dryness, the ammonium salts removed by gentle ignition, water added to the residue, and the insoluble matter filtered off. The filtrate is mixed with a few drops of hydrochloric acid, and evaporated to dryness with an excess of platinum chloride. The residue of double chlorides thus obtained is treated with alcohol in the usual manner, but the insoluble potassium compound, instead of being weighed, is ignited at a low temperature, the residue exhausted with boiling water, and the resulting solution of potassium chloride titrated with standard silver nitrate. The alcoholic solution containing the excess of platinum chloride and the platinum sodium chloride is evaporated to dryness in a platinum basin, sufficient ammonium chloride solution added to combine with all the platinum, the mixture evaporated to dryness, and the residue cautiously ignited. The final residue is extracted with hot water, and this solution also titrated with silver nitrate. From the results of the two titrations the respective amounts of potassium and sodium are calculated.

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* *Journ. Amer. Chem. Soc.*, 1897, p. 388.

† *Loc. cit.*, p. 33.

‡ *Chem. News*, 1890, lxi. p. 291.

Estimation of Sulphur.—The finely divided flesh is placed in a large silver or nickel dish, and covered with about twice its weight of sodium carbonate, on which is laid a piece of sodium hydroxide about half the weight of the carbonate. The dish is moved slowly over a small flame until all evolution of gas has ceased, and a half fused mass is obtained. Finely powdered sodium peroxide is then dusted over in successive small quantities until the carbon has been completely burned away. When cold the mass is treated with water, the solution filtered, hydrochloric acid containing bromine added, and the liquid boiled until free from odour. The sulphate is then precipitated with barium chloride in the usual manner.*

Estimation of Chlorine.—A weighed quantity of the flesh is calcined with calcium nitrate, the residue dissolved in hot dilute nitric acid, and the chlorine determined either gravimetrically or volumetrically.

Estimation of Phosphoric Acid.—J. Katz † lays stress on the importance of determining the amount of this constituent. He states that the phosphoric acid which can be extracted from the flesh with water belongs to the phosphates, whilst that obtained from substances soluble in alcohol is a constituent of the lecithin. The substances insoluble in both solvents contain the phosphorus of the nucleïns (see p. 21).

Estimation of the Total Nitrogen.—The total nitrogen may be determined either by combustion with soda-lime, or, more readily, by modifications of Kjeldahl's process.

In ordinary cases correct results are obtained by the Gunning modification, in which the substance is oxidized with boiling sulphuric acid, to which potassium bisulphate and a drop of metallic mercury are added after frothing has ceased.

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* A. von Asboth, *Chem. Zeit.*, 1895, xx. p. 2040; C. Glaser, *Journ. Amer. Chem. Soc.*, 1898, xx. p. 130.

† *Archiv. ges. Phys.*, lxiii. p. 1.

When, however, nitrates are present in any quantity, as in the case of meat which has been pickled in a solution containing nitre, Iodlbauer's modification should be employed. In this 2 grammes of salicylic acid (or phenol) are previously dissolved in the sulphuric acid, and 1 or 2 grammes of zinc dust and a little mercury introduced into the flask before the heat is applied.

Dyer * recommends the rapid introduction of the oxidizing agents, in order to avoid loss through the formation of lower oxides of nitrogen.

Rivière and Bailhache † made experiments with various substances with the object of shortening the time required for the oxidation. They found sodium pyrophosphate, prepared by calcining the ordinary phosphate, the most suitable substance for raising the temperature of the sulphuric acid and accelerating its action. At the same time a much smaller quantity (2 grammes) was required than in the case of potassium bisulphate. The comparative table published in the original paper shows that this modification gives accurate results with horn, dried blood, and flesh.

The Soluble Extract and Residual Muscular Fibre.—König adopts the following method of determining the soluble substances in flesh:—About 50 grammes of the flesh, freed as completely as possible from fat, are repeatedly extracted with cold water, the united extracts filtered, the filtrate made up to definite volume, and aliquot portions taken for the subjoined estimations:—

(i.) *Total Soluble Matter.*—An aliquot portion is evaporated in a platinum basin and dried at 100°–105° C.

(ii.) *Ash.*—The residue left on evaporation of the water is ignited in a covered platinum basin and weighed. About 94 per cent. of the total mineral matter in the flesh goes into solution.

(iii.) *Total Soluble Nitrogen.*—An aliquot portion is evaporated in a tinfoil basin, the residue (with the tin-foil) introduced into a Kjeldahl flask, and the nitrogen determined in the usual way.

(iv.) *Soluble Albumin.*—An aliquot portion is boiled, the coagulated albumin filtered off, and the amount of nitrogen remaining in the filtrate determined and deducted from the total nitrogen. The difference, multiplied by 6·3, gives the quantity of albumin.

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* *Analyst*, 1895, p. 242.

† *Bull. Soc. Chim.*, 1896, xvi. pp. 806–811; *Analyst*, 1896, p. 267.

Collagene.—On treating flesh with boiling water instead of cold, or at 40° C., no albumin is dissolved, but gelatin derived from the connective tissue passes into solution. A weighed quantity of the flesh is first extracted with cold water, as above, and then with boiling water, which dissolves the collagene, leaving behind the fat and fibre. The amount of collagene is determined by evaporating an aliquot part of the hot water extract and drying the residue at 100°–105°.

Muscular Fibre.—The residue left from the successive cold and hot water extractions is collected on counterpoised filter-papers, washed with hot water, then with warm alcohol, to remove the water, and finally extracted with ether, which removes nearly all the fat. It is then dried at 105°–110° C. and weighed.

The amount of the constituents of flesh soluble in water varies between 4 and 8 per cent., the mean, including albumin and gelatin, being from 6 to 6·5 per cent.

Substances Soluble in Alcohol.—The amount of these is determined in the same manner as the aqueous extract. According to König they consist of flesh bases, non-nitrogenous extractives and salts, and vary in quantity from 1·5 to 3 per cent., the mean being about 2 per cent. The strength of the alcohol used is 80–90 per cent.

Nature of the Soluble Nitrogenous Substances.—Salkowski [*] treats the flesh with water at a temperature not exceeding 30° C., in order to prevent as completely as possible the gelatin dissolving. He finds that under these circumstances 22·6 per cent. of the total nitrogen of the flesh is dissolved, the solution containing coagulable albumin, albumoses, peptones, the flesh bases, and Siegfried's carno-phosphoric acid.

Determination of Phospho-Carnic Acid.[†]—After precipitating the phosphoric acid from the extract by means of calcium chloride and ammonium hydroxide, the carno-phosphoric acid is precipitated with ferric chloride. The precipitate, which also contains some ferric hydroxide, is dried, and the nitrogen it contains determined by Kjeldahl's process. The quantity of nitrogen multiplied by 6·124 gives the carno-phosphoric acid.

Determination of the Amide Nitrogen.—Of the numerous methods which have been proposed for the estimation of amide nitrogen in the presence of proteids, J. W. Mallet [‡] has found precipitation of the proteids, with phosphotungstic acid supplemented in some cases by precipitation with tannin, to give the most satisfactory results.

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[*] *Forschungs Berichte*, 1897, p. 22.

[†] Balke and Ide, *Zeit. Physiol.*, 1896, p. 330.

[‡] *Bull.* 54, *U.S.A. Department of Agriculture*, abst. *Analyst*, 1899, p. 328.

For the analysis of raw or cooked meat he triturates a weighed quantity with sharp-edged sand (previously ignited) or with hard glass, so as to thoroughly subdivide the tissue. Two aliquot parts of this pulp are taken, of which one is used for the determination of the total nitrogen. The other is digested with cold water (to avoid formation of gelatin) so long as soluble matter is removed to any extent. By this treatment kreatinine and sarcosine are readily dissolved; kreatine fairly readily; but xanthine, hypoxanthine (1 : 300) and carnine (1 : 312) are less soluble.

The filtrate is rendered slightly acid with acetic acid, heated to about 99° C., and filtered from any precipitate.

An acidified solution of phosphotungstic acid is added to this filtrate so long as a precipitate results, any large excess being avoided. A little powdered glass or sand is then added, the contents of the beaker heated to about 90° C., and filtered, and the precipitate washed with water, also at about 90° C.

Assuming that only proteid and amide nitrogen are now present, the former is determined by Kjeldahl's method in the precipitates and deducted from the total nitrogen previously determined.

When peptones are present they are incompletely precipitated by phosphotungstic acid, and the solution should therefore be treated with tannic acid (5 to 10 per cent. solution) and filtered before the addition of the phosphotungstic acid, the nitrogen of the precipitate being estimated and added to the proteid nitrogen.

As factors for the conversion of the nitrogen found in the proximate constituents, Mallet prefers the following:—

For proteids and allied substances, multiply the nitrogen by 6·25.
For flesh bases and simpler amides of animal origin, multiply by 3·05.
For simple amides and amido acids of vegetable origin, multiply by 5·15.
For mixed amido-constituents of unabsorbed solid residues in digestion experiments, multiply by 9·45.

The solution of phosphotungstic acid used is a 5 to 10 per cent. solution in 2·5 per cent. hydrochloric acid.

Estimation of the Fat.—After removing all visible fat from flesh, the muscular tissue still contains a considerable proportion, which it is not easy to extract completely.

Dormayer * shows that even after extracting dried and powdered flesh for five months with ether, fat is still retained by the tissue. He recommends digesting the flesh with an acid solution of pepsin, and extracting the fat with ether from the solution thus obtained.

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* *Vierteljahrsch. f. Nahr. u. Genussm.*, 1895, p. 325.

O. Franke[*] steeps 20 grammes of the finely-divided flesh in 100 c.c. of 96 per cent. alcohol for twenty-four hours, with frequent stirring. The alcohol is then drained off, and the treatment repeated twice or thrice with the same quantity of absolute alcohol, and then twice with ether. The residue, freed from ether on the water-bath, is finely powdered and extracted for twenty-four hours, first with ether, and then with petroleum spirit (b. p. 60° C.).

E. Voit[†] gives a simpler method. 100 grammes of the finely-divided flesh are mixed with sufficient alcohol to form a pasty mass, which is dried, with frequent stirring, on the water-bath, in which the water is kept below 80° C. About fifteen hours are required for this. The dried substance is then powdered and passed through a sieve, with a mesh of 0·4 mm. Four grammes of the flesh thus prepared are dried at 70° C. for twelve hours and extracted with ether for twenty-four hours in a Soxhlet apparatus. The crude fat is dissolved in petroleum spirit, the solution filtered and evaporated, and the residue weighed.

The flesh after extraction, tested by Dormayer's digestion method, still contains from 0·59 to 1·7 per cent. of fat, but Voit affirms that the fat obtained by continuing the digestion for longer than twenty-four hours is much less pure, and that the final product of Dormayer's method is also very impure.

A rapid process has recently been described by *Liebermann and Szekely.*[‡] Five grammes of the minced flesh are boiled for thirty minutes with 30 c.c. of a 50 per cent. solution of potassium hydroxide (sp. gr. 1·54) in a flask of the following description:—The body is 7·5 cm. in diameter and 5·5 cm. deep, and has a flat bottom. The neck is 19·5 cm. long and 3·5 cm. in diameter throughout its length. When filled to about the middle of the neck the flask holds about 290 c.c., and has a mark at 240 c.c.

After the boiling the contents of the flask are cooled, mixed with 30 c.c. of 90 to 94 per cent. alcohol, and again heated for about ten minutes. When cold, 100 c.c. of 20 per cent. sulphuric acid (sp. gr. 1·145) are cautiously added, with constant shaking and continual cooling, in order to avoid a possible loss of volatile fatty acids. The liquid, which finally contains an excess of about 4·4 grammes of sulphuric acid, is mixed with 50 c.c. of petroleum spirit (sp. gr. 0·6 to 0·7 and boiling-point about 60° C.), and the flask closed with a rubber cork, and shaken about thirty times at intervals of one or two minutes. A saturated solution of sodium chloride is then added, so that the flask is filled to about the middle of the neck, whilst the aqueous layer below the petroleum spirit stands at the mark (240 c.c.).

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[*] *Zeit. f. Biol.*, 1897, pp. 549–554.

[†] *Zeit. f. Biol.*, 1897, pp. 555–582.

[‡] *Chem. Zeit. Rep.*, 1898, xxii. p. 288.

After again being shaken once or twice the closed flask is placed in a vessel of water and allowed to stand at not too high a temperature. As soon as the petroleum layer (which now contains the entire fatty acids in solution) has separated, 20 c.c. are pipetted off into a wide-necked flask of about 150 c.c. capacity, mixed with about 40 c.c. of neutral 96 per cent. alcohol, and titrated with decinormal alcoholic potassium hydroxide. The titrated liquid is then transferred to a weighed glass basin, holding about 80 c.c., and provided with a ground glass cover, in which it is cautiously evaporated to dryness on the water-bath at a low temperature, and finally dried for an hour at 100° C., and weighed.

In order to calculate the amount of fat, the potassium in the soap must be deducted and the equivalent amount of glycerin radicle added. One c.c. of decinormal potassium hydroxide = 0·00391 gramme of potassium and 0·00136 of (C_3H_5), so that one must subtract as many times 0·00255 gramme as the number of c.c. of alkali used in the titration. The quantity of fat in the flesh can thus be calculated by the formula:—

$$F = \left[\frac{S - 0{\cdot}01 - (K \times 0{\cdot}00255)}{a}\right] . \quad 250$$

in which F is the percentage of fat required; S the weight of potassium soap from 20 c.c. of the petroleum spirit; K the number of c.c. of decinormal potassium hydroxide; and a the weight of the substance under examination.

According to *O. Polimanti*[*] the following simple method gives practically the same results as the Dormayer digestion process. Two grammes of the powdered flesh are shaken for six hours with 200 c.c. of ether and 2 c.c. of metallic mercury, and the fat determined in an aliquot portion of the filtered extract.

THE DIGESTIBILITY OF DIFFERENT KINDS OF FLESH.

As the conditions which exist in artificial digestion experiments are very different from those of the natural process it is not wise to base too general conclusions on the results of such experiments. Nevertheless, they may often furnish valuable information as to the behaviour of flesh under the influence of one or more of the many factors which go to form the physiological processes which we term digestion.

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[*] *Pflüger Archiv*, 1898, lxx. 366.

Artificial Peptic Digestion Experiments.—A simple method of obtaining the gastric juice for such determinations is to cut the fresh mucous membrane of a pig's stomach into small pieces, and to mix the fragments with 5 litres of water and 100 c.c. of 10 per cent. hydrochloric acid. After adding a small quantity of an alcoholic solution of thymol as a preservative, the mixture is left for twenty-four hours, with occasional agitation, and is then filtered, first through flannel, and then through paper. Finally the degree of acidity is determined and brought to exactly 0·2 per cent. As thus obtained the solution of gastric juice can be kept unchanged for months.

One of the dried pepsins in the market may be used instead of the freshly prepared gastric juice in the proportion of about 0·5 gramme to 100 c.c. of dilute hydrochloric acid (0·2 per cent.).

Five grammes of the lean meat, in as fine a state of division as possible, are mixed in a flask with 500 c.c. of the artificial gastric juice, and the flask immersed in a water-bath maintained at a constant temperature of 40° C. for three hours. The portion remaining undissolved is then collected on a filter, washed, dried, and weighed, an allowance being made for the amount of water contained in the original flesh.

Artificial Pancreatic Digestion Experiments.—For the preparation of artificial pancreatic juice a portion of the pancreas of an ox may be well triturated with sand in a mortar, and extracted with cold water, or with a 2 per cent. solution of sodium carbonate. Thymol should be added to the extract as a preservative, as in the case of artificial gastric juice.

The digestion experiments are made in the same way as those with pepsin, but the liquid instead of being acid should be slightly alkaline (1 per cent. of sodium carbonate).

The products formed by the action of pepsin and trypsin on the proteids of flesh are referred to in a subsequent chapter (p. 179).

Comparative Digestibility of Flesh.—Chittenden and Cummins * have determined the relative digestibility of different kinds of flesh by pepsin. In each case 20 grammes of the sample were freed as completely as possible from sinew, fat, skin, and bone, and treated with 5 grammes of pepsin dissolved in a litre of hydrochloric acid (0·2 per cent.).

The amount of cooked beef digested was 4·0461 grammes, and this was taken as the standard.

Representing this amount as 100, the relative digestibility in artificial gastric juice of other kinds of flesh under the same conditions were:—

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* *Amer. Chem. Journ.*, vi. p. 318.

Veal,	94·89	Mackerel,	86·24
Mutton,	92·15	Herring,	82·34
Lamb,	87·93	Shell-fish,	82·5
Hen (light flesh),	86·72	Eel,	71·76
,, (dark flesh),	84·42	Lobster (female),	79·06
Salmon,	92·29	,, (male),	69·0
Trout,	87·03	Crab,	67·13
Cod,	72·39	Frog's leg,	80·40

The digestibility of raw beef as compared with cooked beef was as 142·38 is to 100.

Physiological Experiments.—In addition to artificial digestion experiments many physiological experiments have been made on animals and human beings, weighed quantities of flesh or other food being given, and the amount and nature of the excreta determined, the difference being regarded as digested.

Thus, in 1862, Ranke showed that in a feeding experiment in which 18·32 grammes of beef were given, 11·5 per cent. of the total nitrogen was found in the waste products.

W. Atwater * made experiments on these lines to determine the digestibility of shell-fish in comparison with beef. Of the former about 1550 grammes, and of the latter about 1200 grammes, were eaten by a young man, together with a certain proportion of butter, salt, and spice.

The results thus obtained were:—

	Absorbed.				Separated in Excreta.			
	Dry Substance.	Nitrogenous Matter.	Fat.	Salts.	Dry Substance.	Nitrogenous Matter.	Fat.	Salts.
Fish,	95·1	98·0	91·0	77·5	4·9	2·0	9·0	22·5
Beef,	95·7	97·5	94·8	78·5	4·3	2·5	5·2	21·5

From these it follows that, in the case of this individual at least, shell-fish is as digestible as beef, and this conclusion received confirmation in similar experiments on a dog.

But since the amount of a given food which is capable of being absorbed into the system varies considerably in the case of different individuals, a conclusion as to the absolute degree of digestibility can only be drawn from such experiments when they have been tried with a very large number of people.

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* *Zeit. f. Biol.*, 1887, xxvii. p. 215.

CALCULATION OF THE FOOD VALUE OF FLESH.

This ought to be based not only on the amount of nutriment contained in the flesh, but also on the amount capable of being absorbed and on the effect of the flavour. But inasmuch as the two last factors vary with each individual it is only possible to calculate the value approximately from the first, and to regard that food as the cheapest which contains the most nourishment.

König adopts the method proposed by Emmerich, and starts from the fact that the actual market value of nitrogenous substance (proteid) is higher than that of fat, and that carbohydrates are cheaper than fat.

If 1 gramme of carbohydrate be taken to represent 1 nutrient unit in value, 1 gramme of fat, and of proteid represent 3 and 5 nutrient units respectively.

Thus, for example, if 1 kilo. of beans cost 8d.,

Proteids, .	230 grammes	× 5 =	1150 nutrient units
Fat, . .	20 „	× 3 =	60 „
Carbohydrates,	535 „	× 1 =	535 „
			1745

Here the price of 1 food unit of beans would equal $\frac{8}{1745}$d.

The following figures taken from König's table illustrate this method:—

	Water.	Proteids.	Fat.	N.-free Extractives.	Ash.	Sum of nutrient units per kilo.
Mutton, very fat,	47·91	14·80	36·39	0·05	0·85	1832·2
Sheep's tongue, .	67·44	14·29	17·81	0·09	1·00	1230·8
Sheep's liver, .	69·30	21·64	4·98	2·73	1·35	1258·7

F. Strohmer* gives the subjoined table of the food value of different kinds of flesh calculated by this method:—

	Per Cent.			Nutrient Units per kilo.
	Proteids.	Fat.	Carbo-hydrates.	
Beef, moderately fat, .	21·0	5·5	...	1215
,, lean, . . .	21·0	1·5	...	1095
Veal, ...	20	4·0	...	1120
Pork, fat, . . .	14·5	37·5	...	1845
,, lean, . .	20·0	7·0	...	1210
Lard, . . .	0·3	99·0	...	2985
Bacon, . . .	5·0	78·0	...	2590
Game, . . .	22·5	1·0	...	1155
Rabbit, . . .	21·5	10·0	...	1375
Heart, . . .	18·0	8·0	...	1140
Kidney, . . .	18·5	4·0	...	1045
Liver, . . .	20·0	4·0	...	1120
Salt Herring, . .	19·0	17·0	...	1460
Liver Sausage, . .	11·0	14·5	21·0	1006
Blutwurst (Blood Sausage), .	10·0	9·0	20·0	790

According to Lehmann † this empirical method furnishes results which agree fairly well with the actual relation in price.

SCHEME FOR THE EXAMINATION OF FRESH MEAT.

The following outline for a preliminary examination of raw meat may be serviceable:—

1. **The Colour.**—Normal.—White, as in lamb or veal; reddish, as in beef and mutton.
   Abnormal.—Melanosis, yellow, white, due to disease; xantosis, dark purple (acute fever), reddish brown (CO_2 poisoning), scarlet (CO poisoning or bacterial deposit). Iridescence, gray, violet, green (incipient decomposition) (*cf.* pages 70–72).
2. **The Consistency.**—A skewer forced into the flesh should meet with equal resistance throughout. The opposite case may denote decomposition or the presence of abscesses. When cut with a knife the division should be even and regular (*cf.* pages 72 and 77).
3. **The Odour.**—See pages 73 and 78.
4. **The Fat.**—This should be present in suitable proportions. Extreme leanness denotes disease (*cf.* pages 72 and 289).

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* *Die Ernahrung des Menschen*, p. 324.

† *Methods of Practical Hygiene*, p. 413.

5. Reaction of the Meat Juice towards Litmus.—Acid denotes sound meat or acid decomposition. Alkaline denotes decomposition or presence of alkaline salts as preservatives (*cf.* page 74).
6. Eber's Test for Putrefaction.—This is carried out as described on page 75.
7. Degree of Moisture.—Abnormal moisture, as visible to the eye, is a symptom of several diseased conditions (*cf.* page 79).
8. Frozen Meat is detected by the appearance of the meat juice to the naked eye and under the microscope (page 104).
9. 'Blown' Meat.—The general characteristics are described on page 77.
10. Meat treated with Antiseptics.—(*a*) Test for decomposition with litmus and Eber's test (pp. 74–75); (*b*) Examine the meat fibres under microscope for decomposition and result of action of sulphites (pp. 117 and 121); (*c*) Test the meat juice for borates with turmeric paper (p. 119); (*d*) Test for salicylic acid (p. 122), and formaldehyde (p. 123).

CHAPTER V.

METHODS OF EXAMINING ANIMAL FAT.

Methods of Examining the Fat.—In the present writer's opinion the analytical methods described in the following pages will be found among the most suitable for the examination of the fat obtained by the methods described in the preceding chapter. For various modifications of these, and for alternative processes, the reader is referred to works dealing specially with fats and oils.

Crystallization from Ether.—This may sometimes give an indication as to the nature of the fat. Pigs' fat, excepting that from the flare, is deposited in characteristic crystals with chisel-shaped ends, while beef-fat, mutton-fat, and horse-fat give fan-like bunches of needle-shaped crystals. Hehner and Mitchell * have shown that the form of the crystals depends on the proportion of stearic acid in the fat, and that on continued recrystallization of a lard which at first gives the broad-ended crystals, the deposits become more and more rich in stearic acid, and eventually assume the form of the crystals from beef-fat.

Specific Gravity.—Accurate results are most readily obtained by the use of a Sprengel U-tube.

Melting Point.—The method adopted by the Association of Bavarian Chemists consists in drawing the melted fat into a thin capillary tube, sealing one end, and leaving it for twenty-four hours. The tube is then tied to the stem of a thermometer, which is gently heated in a glycerin bath. The temperature at which the fat becomes perfectly clear and transparent is regarded as the melting point.

Solidifying Point of the Fatty Acids.—The fatty acids are melted in a test-tube and allowed to cool slowly until signs of incipient solidification appear, when they are stirred with a thermometer, graduated in fifths of a degree three times to the right and three times to the left. At a certain stage after this the mercury in the thermometer ceases to fall, and then suddenly rises, often as much as half a degree, and remains stationary for a short time before commencing to fall again. This stationary point is taken as the solidifying point, and is also known as the Dalican 'titre.' By using the same apparatus and details of procedure concordant results are readily obtained by this method.

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* *Analyst*, 1896, p. 329.

The Iodine Value.—This indicates the percentage of iodine or other halogen, calculated into its equivalent of iodine, absorbed by a fat.

Hübl Process.—The method which has hitherto been most widely employed is that of Hübl, varied in the details of working by various chemists. The following is an outline of a method of procedure which, in essential particulars, is the same as that of Hübl:—

Two solutions are prepared: (1) containing 25 grammes of iodine in 500 c.c. of pure 95 per cent. alcohol (or rectified spirit), (2) containing 30 grammes of mercuric chloride in 500 c.c. of the same solvent.

About 0·3 gramme of liquid fat, or 0·8 gramme of solid fat are dissolved in 10 c.c. of chloroform in a stoppered bottle. To the solution are added (1) 20 c.c. of the iodine solution, and (2) 20 c.c. of the mercuric chloride solution. Simultaneously a blank determination is made, the same quantity of chloroform and of the solution being placed in a stoppered bottle containing no fat.

After three hours 10 c.c. of a 10 per cent. solution of potassium iodide is added to each bottle, and the liquid in each titrated with recently standardized sodium thiosulphate, starch paste being used as indicator.

The difference between the blank determination and the other gives the amount of iodine absorbed by the quantity of fat taken.

Care must be taken that there is always an excess of iodine during the absorption.

From Wijs' experiments * it appears that the results are more accurate if the blank be titrated before the absorption rather than after it, and also that seven hours is a somewhat better time limit than three hours.

Wijs' Method.—Recently Wijs' † has thrown light on the nature of the reactions which take place on mixing the Hübl solutions and adding them to a fat, and has shown that the substance chiefly concerned in the absorption of the iodine is hypoiodous acid (HIO), formed by the action of the water present on the iodine chloride derived from the double decomposition between the iodine and the mercuric chloride.

As this acid is extremely unstable, he has devised a means of obtaining it under such conditions as largely prevent its decomposition. This is effected by preparing it by the action of water on iodine chloride ($ICl + H_2O = HCl + HIO$), a solvent being chosen which contains only so much water as will decompose nearly the whole of the iodine chloride, and which at the same time will not be oxidized by the hypoiodous acid. A solution of iodine chloride in 95 per cent. acetic acid fulfils these conditions.

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* *Analyst*, abst., 1899, p. 95.

† *Zeit. angew. Chem.*, 1898, p. 291.

This is prepared by dissolving 13 grammes of iodine in a litre of acetic acid, titrating the solution with standard thiosulphate, and passing a current of chlorine through, until the quantity of thiosulphate required is doubled. With a little practice this point can be readily found by the change in colour.

The solution thus obtained is fairly stable, and is used in the same way as the mixed Hübl solutions, with the exception that the length of time required for the absorption is very greatly reduced, the addition being complete in three or four minutes in the case of fats and oils with low iodine values, while not more than ten minutes are necessary with oils with high iodine values.

The Bromine-Thermal Method.—This method, devised by Hehner and Mitchell,* affords a rapid means of determining the iodine value. It depends on the facts that on adding bromine to a fat or oil a considerable amount of heat is liberated, and that this heat is proportional to the degree of unsaturation.

One gramme of the fat or oil is dissolved in 10 c.c. of chloroform or carbon tetrachloride in a test-tube packed with non-conducting material in a beaker, or preferably in a vacuum-jacketed tube.† A delicate thermometer (graduated in fifths or tenths of a degree) is inserted, and the temperature observed. One c.c. of bromine, previously brought to the same temperature as the chloroform solution, is then introduced, and a note made of the highest temperature reached. The difference between the initial and the final temperatures is the 'bromine-heat value.'

By accurately determining the bromine-heat value and the iodine value of a number of edible fats and oils a ratio can be worked out between the two, so that subsequently it is only necessary to determine the bromine-heat value and to multiply it by the factor, in order to obtain the iodine value. Of course the same apparatus and method of working must alone be used or the factor will be a different one.

The Saponification or Köttstorfer Value.—This indicates the amount of potassium hydroxide, in milligrammes, required to exactly convert the fatty acids in 1 gramme of a fat into the potassium salts, with complete liberation of the glycerin.

Hot Saponification.—From 1·5 to 2 grammes of the fat are mixed in a flask with an accurately measured excess of standard alcoholic potassium hydroxide, and heated on a boiling water-bath under a reflux condenser for about thirty minutes. The liquid is then titrated back with semi-normal acid, with phenol-phthalëin as indicator. The alcoholic alkali is prepared by dissolving about 30 grammes of potassium hydroxide in a little boiling water, making up the solution to a litre with purified alcohol, and filtering it after standing for twenty-four hours.

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* *Analyst*, 1895, p. 146.

† These may be obtained from F. Müller, Holborn.

*Cold Saponification.**—From 3 to 4 grammes of the fat are dissolved in 25 c.c. of petroleum spirit and the solution mixed with 25 c.c. of standardized alcoholic potassium hydroxide (containing as little water as possible). The saponification is usually complete in a few hours, but it is advisable to allow the flask to stand overnight before titrating back the excess of alkali.

Hehner Value.—This shows the percentage of insoluble fatty acids contained in a fat or oil.

From 3 to 4 grammes of the fat are weighed into a small evaporating dish, where they are mixed with 1 to 2 grammes of potassium hydroxide and 50 c.c. of alcohol, and heated on the water-bath until completely saponified. The soap is evaporated to a pasty consistency and dissolved in about 150 c.c. of boiling water, the fatty acids liberated by adding hydrochloric or sulphuric acid, and the flask heated on the water-bath until they melt and form a clear layer on the surface. The contents of the flask are then poured on to a filter of thick paper, previously dried at 100° C., and weighed, and the insoluble fatty acids left on the filter are washed with boiling water until the filtrate ceases to redden litmus. The filter-funnel is then immersed in cold water, which generally causes the fatty acids to solidify. The water is drained off, and the filter and its contents dried at 100° C. in a beaker of known weight. The weighings, taken after two hours', and again after 4 hours' drying, usually agree within a milligramme.

The Reichert Value.—This indicates the definite proportion of volatile fatty acids obtained from 2·5 grammes of a fat by Reichert's distillation process.

Two and a-half grammes of the purified and filtered fat are weighed into a small flask, fitted with a cork through which passes a short piece of glass-tubing, and saponified by adding 5 c.c. of pure alcohol and 6 c.c. of a concentrated aqueous solution of potassium hydroxide (free from carbonate) and heating on the water-bath for a short time. After expelling all traces of alcohol the dry soap is dissolved in 70 c.c. of boiling water, and the fatty acids liberated by adding 5 c.c. of sulphuric acid of the right strength to neutralize the alkali. A few pieces of pumice are introduced into the flask to prevent bumping, and the liquid gently distilled until exactly 50 c.c. of liquid have passed over. This distillate is filtered, the filter washed with boiling water, and the filtrate and washings titrated with decinormal solution of potassium or barium hydroxide. The number of c.c. required is the Reichert value.

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* R. Henriques. *Zeit. angew. Chem.*, 1895, p. 721; 1896, p. 221. *Analyst*, 1896, pp. 67 and 192.

In the Reichert-Meissl process 5 grammes of the fat are used, and the number obtained is considerably higher than the Reichert value.

The Acetyl Value.—This indicates, among other things, the amount of hydroxylated fatty acids present in a fat.

The method which gives the most reliable results is that of Lewkowitsch,[*] who defines the value as the number of milligrammes of potassium hydroxide required to neutralize the acetic acid obtained by saponifying 1 gramme of the acetylated fat.

The glycerides of any hydroxylated acids present are converted into their acetyl compounds by boiling the filtered and purified fat for two hours with an equal volume of acetic anhydride. The oily product is boiled with successive portions of water until the latter has no longer an acid reaction, and is then freed from water and filtered.

From 2 to 4 grammes of the acetylated fat are saponified with a definite volume of standard alcoholic potassium hydroxide, the alcohol evaporated and the soap dissolved in boiling water. The amount of acetate present is then determined by either a distillation or a filtration process.

In the former an excess of sulphuric acid is added, from 500 to 700 c.c. of the liquid distilled by blowing a current of steam through the flask, and the distillate titrated with standard alkali.

In the filtration process, a quantity of sulphuric acid exactly equivalent to the alcoholic potassium hydroxide used is added, the liquid warmed, the layer of fatty acids filtered off and washed with boiling water, and the filtrate and washings titrated with standard alkali.

Free alcohols will also be saponified, and, if present in any quantity, a correction must be made for them. A correction is also necessary when the fat contains any considerable proportion of volatile fatty acids (high Reichert value).[†]

The Acid Value.—This indicates the amount of free fatty acids present in a fat.

A weighed quantity of the fat is mixed with neutral alcohol, heated on the water-bath until the alcohol boils, and titrated with a standard solution of potassium hydroxide. The number of milligrammes of potassium hydroxide required to neutralize the free fatty acids in 1 gramme of fat gives the acid value.

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[*] *Jour. Soc. Chem. Ind.*, 1897, xvi. pp. 503-506.

[†] The meaning of the acetyl value in fat-analysis is exhaustively discussed in a recent paper by Lewkowitsch (*Analyst*, 1899, p. 399).

DETERMINATION OF INDIVIDUAL CONSTITUENTS.

Separation of Liquid and Solid Fatty Acids.—*Treatment of the Lead Soaps with Ether.*—Several methods[*] have been based on the fact that the lead salts of the unsaturated fatty acids are much more soluble in ether than those of the solid fatty acids (Varrentrapp). In each case there is only a fractional separation or concentration, and the portion soluble in ether, although very much richer in liquid fatty acids, still contains solid fatty acids, while the insoluble portion is not free from liquid fatty acids. As, however, it is possible, by working under exactly the same conditions, to obtain concordant results, the method in one or other of its modifications is widely employed, and often affords valuable information.

The following process is essentially that of Rose:—One gramme of the mixed fatty acids is placed in a stoppered flask with 0·5 gramme of lead oxide, and about 80 c.c. of ether, and after standing for twenty-four hours, with an occasional shake, the liquid is made up to 100 c.c. with ether, the flask well shaken, and the insoluble matter allowed to settle. Twenty-five c.c. of the ether are then withdrawn by means of a pipette, the end of which is covered with a porous plug of cotton-wool, to serve as a filter. The solvent is evaporated and the residue dried in a current of carbon dioxide, and weighed. The lead it contains is then determined by adding 2·5 c.c. of dilute sulphuric acid (1 : 5), digesting on the water-bath, adding 40 c.c. of 95 per cent. alcohol, collecting the lead sulphate on counterpoised filters, washing it with alcohol, drying and weighing. From the percentage of lead in the dried sulphate the proportion of fatty acids which were in combination with it in the residue may be calculated, and also their molecular weight.

Determination of the Iodine Value of the 'Liquid' Fatty Acids.—Fifty c.c. of the ethereal solution are withdrawn from the flask with the filter pipette and shaken with dilute hydrochloric acid in a separating funnel, in order to liberate the fatty acids; the ethereal layer is washed with successive portions of water until free from chloride, after which 25 c.c. are withdrawn and evaporated to dryness in a weighed flask, and the iodine value of the residue determined in the usual manner. From the time of the liberation of the fatty acids the greatest care is necessary, to

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[†] Oudemans, *J. prak. Chem.*, 99, p. 407; Muter and De Koningh, *Analyst*, 1889, p. 61: Kremel, *Pharm. Centralb.*, 5, p. 337; Rose, *J. Soc. Chem. Ind.*, 1887, p. 306.

prevent their oxidation, and throughout the washing and evaporation the air in the separating funnel and the flask must be replaced by carbon dioxide.

(ii.) *Treatment of the Lead Soap with Benzene.*—Finding that the lead salts of the unsaturated fatty acids were much less soluble in benzene than in ether, Farnsteiner * has devised the following method of separation, in which his experiments with known mixtures show that the proportion of liquid fatty acids obtained are from 1 to 3 per cent. too low, while that of the solid acids is in the maximum 1·65 per cent. too high:—

From 0·6 to 1 gramme of the fat is saponified in an Erlenmeyer flask with alcoholic potassium hydroxide, the solution neutralized with acetic acid, and, after evaporation of the alcohol, the soap dissolved in 100 c.c. of boiling water and precipitated with 30 c.c. of a boiling solution of lead acetate (containing about 1 gramme). When cold the liquid is filtered and the residue in the flask washed with cold water, freed from the latter as completely as possible, and dissolved in 50 c.c. of hot benzene. The solution is left at the ordinary temperature for fifteen minutes, and is then cooled for about two hours at 8° to 12° C. In order to separate the liquid from the crystalline deposit, the flask is closed with a cork, having two holes, through one of which passes a short straight tube, while the other holds a tube reaching to the bottom of the flask and having its exterior end bent downwards outside the flask. The interior opening of the tube is covered with a plug of cotton wool which serves as a filter, and the liquid is driven upwards through this by forcing air into the flask through the short straight tube. When the liquid has been removed as completely as possible in this way, the flask is washed with 10 c.c. of benzene at 10° C., which is similarly expelled. The precipitate is then dissolved in 25 c.c. of hot benzene, again cooled for an hour at 8° to 10° C., and the liquid again filtered. In the same way a third precipitation and filtration are carried out, so that altogether from 120 to 130 c.c. of the benzene filtrate are obtained.

The liquid fatty acids are recovered from the united filtrates by shaking the latter with 10 c.c. of hydrochloric acid, filtering the solution of fatty acids through cotton wool, into a flask, and distilling off the benzene in a current of hydrogen, to prevent oxidation. The solid fatty acids are determined by heating the insoluble lead salts in a flask with 25 to 30 c.c. of benzene for a short time, then adding dilute hydrochloric acid (1:10), continuing the heating for about fifteen minutes under a reflux condenser, washing the solution, and evaporating the benzene.

When free fatty acids are to be examined the best method is to dissolve them in benzene and to heat the solution under a reflux condenser with lead hydroxide, obtained by precipitating a solution of lead acetate with sodium hydroxide, washing the precipitate with water, alcohol, and ether, drying it at a gentle heat, and finely powdering it. The proportions required for one part by weight of solid and liquid fatty acids are 0·4 and 0·2 parts respectively.

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* *Zeit. Nahr. u. Genussm.*, 1898, 1, pp. 390–399.

Determination of the Iodine Value of the Liquid Fatty Acids.—This may be done with the residue of acids obtained in the manner described above, every precaution having been taken to prevent their becoming oxidized. The risk of oxidation is greatly reduced and the manipulation simplified by determining the iodine value of the fatty acids while still in solution in the benzene. Farnsteiner * has found that benzene from which all thiophene has been removed does not absorb a trace of iodine on treatment with Hübl's solution, and on this fact bases the following method:—From 1 to 2 grammes of the fat are converted into the lead salts of the fatty acids in the usual manner, and dissolved in 100 c.c. of benzene (free from thiophene) at a gentle heat. After being left for ten to fifteen minutes, until a precipitate commences to form, the flask is allowed to stand for two hours at a temperature of from 8° to 12° C., and the liquid then filtered without subsequent washing of the precipitate. After shaking the filtrate with about 100 c.c. of dilute hydrochloric acid (1:10), until the fatty acids are liberated, the benzene solution is washed twice with water and filtered. Two portions of 25 c.c. are taken from the filtrate and treated with the Hübl solution in the usual manner, whilst a similar third portion is evaporated in a current of hydrogen, and the residue weighed in order to determine the quantity present in the other fractions.

Benzene † can be freed from thiophene by heating 120 c.c. to the boiling point under a reflux condenser with 5·8 grammes of aluminium chloride, and distilling, care being taken to exclude moisture. The distillate is washed with sodium hydroxide solution and dried with calcium chloride.

Treatment of the Zinc Salts with Ether.—In Jean's method the fat is saponified with alcoholic potassium hydroxide, the excess of alkali neutralized with acetic acid, and the alcohol evaporated on the water-bath. The soap is dissolved in hot water, a hot solution of zinc acetate (1 part to 2 parts of fat) added, and the zinc soap washed with hot water and alcohol, pressed between filter-paper, and extracted with about ten times its volume of anhydrous ether for fifteen to thirty minutes, under a reflux condenser. After cooling, the solution is filtered into a separating funnel, shaken with dilute hydrochloric acid, and the ethereal layer containing the liberated fatty acids, washed with water, and parts of it filtered into weighed flasks, where the ether is evaporated and the iodine value of the residues determined in the usual way. During the filtrations and evaporations every precaution is taken to prevent oxidation.

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* *Zeit. Untersuch. Nahr. Genussm.*, 1898, p. 529.

† Heusler, *Zeit. angew. Chem.*, 1896, p. 750.

Bömer * has recently made experiments on the determination of the iodine value of the zinc salts without previous conversion into fatty acids. He points out that since the molecular equivalents of the higher unsaturated fatty acids differ but slightly, a variation in the percentage of those acids in a mixture would not have a very great influence on the result. Thus 100 parts of oleic acid correspond to 111·19 of zinc oleate; 100 of linolic acid to 111·27 of zinc linoleate; and 100 of linolenic acid to 111·35 of zinc linolenate. Hence, without risk of a considerable error, the molecular equivalent of the zinc salts of mixed liquid fatty acids may be taken as that of the oleic acid salt (627·1); and since 100 parts of zinc oleate correspond to 89·94 parts of oleic acid, the iodine value of the liquid fatty acids (taken as oleic acid) may be calculated by dividing that of the zinc soap by 0·8994 or multiplying it by 1·112.

(iii.) *Treatment of the Fatty Acids with Sulphuric Acid and Extraction of the Saturated Acids with Petroleum Spirit.*†—From 0·5 to 1 gramme of the fatty acids are melted in an Erlenmeyer flask, the flask chilled in ice water, 3 c.c. of 85 per cent. sulphuric acid added, and the temperature allowed to rise. When once the reaction commences a clear solution is rapidly obtained, and the flask is again cooled. Fifty c.c. of petroleum spirit are then introduced, the flask well shaken, the petroleum spirit decanted into a separating funnel, the flask rinsed out twice with 10 c.c. of petroleum spirit, the total extract washed with water, the solvent evaporated, and the residue, consisting of the saturated fatty acids, dried and weighed. ‡

Determination of Stearic Acid.—Hehner and Mitchell § have devised a method of estimating this constituent, in which the fatty acids are crystallised from alcohol previously saturated with pure, or nearly pure, stearic acid, at a definite temperature. The stearic acid is most readily obtained by recrystallising the fatty acids of cocoa butter from alcohol until a product is obtained which melts between 68° and 69° C. An excess of this is dissolved in 95 per cent. alcohol, and the flask kept immersed for twelve hours in ice-water. In the morning the liquid is drawn off by means of a suction filter, without withdrawing the flask from the ice-chest. The filter consists of a thistle funnel covered with a linen cloth, and the method of manipulation is shown in the accompanying figure.

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* *Zeit. f. Untersuch. Nahr. Genussm.*, 1898, p. 541.

† E. Twitchell, *Journ. Soc. Chem. Ind.*, 1897, p. 1002.

‡ According to Lewkowitsch (*Analyst*, 1900, p. 64) this method does not yield quantitative results.

§ *Analyst*, 1896, p. 316.

From 0·5 to 1 gramme of the fatty acids of the fat under examination are dissolved in 100 c.c. of this saturated alcohol, and the flask left immersed in the ice-water in the ice-chest for twelve hours. The liquid is then drawn off through the filter-tube, the flask washed out three times with 10 c.c. of the saturated alcohol at 0° C., each portion being withdrawn in the same manner, and the residue dried at 100° C., and weighed. An allowance, experimentally determined to be 5 milligrammes, is made for the amount of stearic acid contained in the saturated alcohol unavoidably left in the flask. (*Cf.* pp. 54 and 56.)

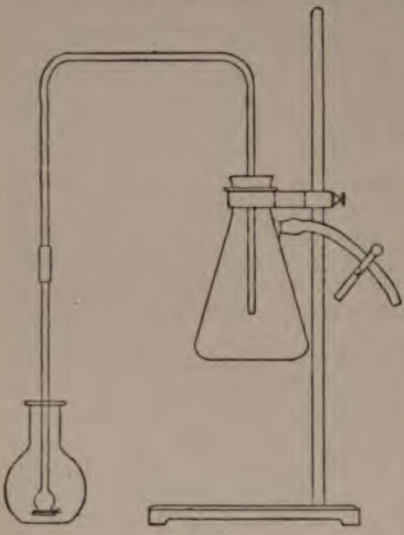

FIG. 17.

Determination of Oleic Acid. — Farnsteiner * has based a method of determining this acid on the insolubility of barium oleate in a mixture of cold benzene and alcohol. The fat is saponified and barium chloride added to the hot soap solution. The precipitate is washed with water and dissolved in 50 c.c. of hot benzene containing 2·5 c.c. of 95 per cent. alcohol. The precipitate, which deposits on cooling, is redissolved in 50 c.c. of benzene containing 10 c.c. of alcohol, and the resulting precipitate again recrystallised from a mixture of 50 c.c. of benzene and 20 c.c. of alcohol.†

The fatty acids recovered from the insoluble salts consist of saturated fatty acids and oleic acid, which can be separated from one another by Farnsteiner's method (p. 97).

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* *Zeit. Untersuch. Nahr. Genussm.*, 1899, pp. 1–27.

† Lewkowitsch has recently shown (*Analyst*, 1900, p. 64) that this method is unreliable. It might be possible, however, to obtain satisfactory results by previously saturating the solvent with pure barium oleate.

The barium salts of the fatty acids can also be prepared directly from the fats by saponifying them with a solution of barium hydroxide in equal volumes of benzene and methyl alcohol.

Determination of Linolic Acid.—According to Farnsteiner it is possible to estimate this acid by taking advantage of the insolubility of its bromide in cold petroleum spirit.

The fatty acids of the oil or fat are dissolved in chloroform, or petroleum spirit, the solvent and excess of bromine evaporated, and the deposit filtered off, washed, dissolved in petroleum spirit, recrystallised, collected, and weighed.

As a rule it was necessary, where only small quantities of linolic acid were present, to brominate the liquid fatty acids (p. 97), or the soluble fatty acids obtained in the separation of oleic acid as a barium salt (p. 100).

In this way lard was found to contain traces of both linolic acid and linolenic acid. Horse fat contained 9·9 per cent. of linolic acid, which was also found in ox-tallow, accompanied by traces of linolenic acid.

Determination of Linolenic Acid.—*Indirect Estimation.*—Hehner and Mitchell* have devised the following method:—On adding bromine to a chilled acetic acid solution of the total liquid fatty acids of an oil, there is an immediate precipitate if linolenic acid be present in more than traces, but this may also contain linolic bromide if it be present in any quantity.

The precipitate is collected on a filter, washed first with cold acetic acid so as to remove oleic dibromide, and then with very cold ether, dried, and weighed. The bromine it contains is determined, and the relative proportion of linolenic hexabromide calculated by means of the formula

$$\frac{63{\cdot}3x}{100} + \frac{(100 - x)53{\cdot}3}{100} = m$$

or

$$x = 10(m - 53{\cdot}3),$$

in which m equal the percentage of bromine found, x the required percentage of hexabromide, and 63·3 and 53·3 the respective percentages of bromine in the pure hexa- and tetra-bromides.

Direct Estimation.—In Farnsteiner's method of estimating linolic acid (*vide supra*) it was found that in some cases the bromides of the liquid fatty acids were not completely soluble in hot petroleum spirit, and that the insoluble residue had the characteristics of linolenic hexabromide.

Hence it seems probable that this may be made the basis of a method of separating linolic and linolenic acids.

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* *Analyst*, 1898, p. 314.

CHAPTER VI.

THE PRESERVATION OF FLESH, AND THE COMPOSITION AND EXAMINATION OF PRESERVED FLESH PRODUCTS.

The Decomposition of Flesh.

THE organic substances which compose the cells of the animal tissues and fluids are, as it were, in a state of unstable equilibrium, a constant series of molecular changes going on, with destruction and reconstruction of the cell materials. So long as the cell is endowed with the force known as 'life,' it is able to resist the disintegrating effect of the numerous micro-organisms which, under suitable conditions, speedily break down complex animal compounds into simpler and more stable bodies. But when once the cell is dead, the process of decay or putrefaction speedily commences, unless means be taken to destroy or render inert the bacteria already present, and to prevent the access of others. The conditions essential for the bacterial decomposition of flesh are the presence of a sufficient quantity of moisture, and a suitable temperature, while the presence of atmospheric oxygen is often an accelerating influence.

The methods adopted in the preservation of meat are based on a consideration of these facts, and for convenience may be considered under the following heads:—1. Preservation by Cold; 2. Drying; 3. Salting; 4. Smoking; 5. Heat-Sterilisation and Exclusion of Air; 6. Antiseptic Agents. Obviously this classification is by no means an exact one, as the divisions overlap one another in many cases.

Preservation by Cold.

This method of preservation is perhaps more extensively employed than any other, especially in Russia, where the climate is favourable for its natural application. Preservation by means of artificial cold is also in general use, and enormous quantities of frozen meat are daily supplied to the markets of London and other large cities.

The numerous methods of cold preservation which have been described are based upon either (1) freezing the flesh and keeping it frozen; or (2) keeping it at a temperature of only a few degrees below zero (C.).

Alterations in Frozen Flesh.—Owing to the slow, continuous action of the sarcolactic acid, meat which has been frozen is often exceptionally tender. On the other hand, owing to the loosening of the intermuscular tissue, bacteria can more readily penetrate into the interior of the thawed flesh, and bring about more rapid decomposition. Considerable care is required in the thawing, since if this be done too suddenly the meat when cooked is often wanting in flavour.

Action of Cold on Bacteria.—Bacteria in general, and especially those which bring about putrefaction, appear to be endowed with extraordinary powers of resistance to the action of cold.

Pictet and Young * exposed cultivations of anthrax bacilli, of *B. subtilis*, and of other bacteria, in wooden boxes, to a temperature of − 70° to − 76° C. for twenty hours, and finally for a long period at − 76° to − 130° C., but did not succeed in destroying their vitality. Colemann and Mickendrick * obtained similar results. In their experiments flesh was kept for at least six hours in hermetically-sealed boxes at temperatures from − 6° to − 130° C., but in every instance the flesh, after being kept at a slightly warm temperature, began to decompose in from ten to twelve hours, though protected from subsequent infection.

But cold, although it does not destroy micro-organisms, prevents their development, or at least does so in the case of the putrefactive bacteria, which at low temperatures are unable to decompose the proteids of flesh. There are, however, certain non-proteolytic bacteria which are capable of developing in frozen meat, and especially in that which is kept at a temperature of 0° C., instead of several degrees lower. To this cause Lafar † attributes the unpleasant flavour sometimes acquired by meat which has been kept in an ice-chamber for several days.

This is confirmed by Popp,‡ who states that in cement-lined storage chambers the walls, when moist, swarm with bacteria, which, when grown on beef-gelatin, produce a mouldy flavour, and he considers them to be the cause of the objectionable flavour frequently developed in stored meat.

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* Ostertag, *Handbuch der Fleischbeschau*, p. 535.

† *Technical Mycology*, p. 213.

‡ *Zeit. Fleisch u. Milch Hyg.*, 1898, p 33.

The Detection of Frozen Meat.—When fresh blood is exposed to a temperature of from 10° to 15° below zero (C.), it solidifies, and, when examined under the microscope, shows ruptured corpuscles. This was first described by G. Pouchet in 1866, and has been applied by Maljean * to the recognition of frozen meat. A drop of the blood or meat-juice is expressed from the meat on to a glass slide, covered with a thin glass, and placed under the microscope as rapidly as possible in order to avoid solidification. The juice from fresh meat shows numerous red corpuscles of normal colour and shape floating in a colourless serum, whereas the corpuscles in the drop taken from the frozen meat are all more or less distorted in form, and completely decolorised, whilst the surrounding liquid has a relatively dark colour.

A difference is also apparent to the naked eye, the juice from fresh meat being more abundant and of a redder tint than that from frozen meat. On placing a fragment of the frozen meat in a test-tube containing some water, the liquid becomes coloured much more rapidly and intensely than when fresh meat is used.

Preservation by Drying.

During the process of drying in the sun or by artificial heat flesh loses a large proportion of its water, so that in the finished product one of the essential conditions for the development of bacteria is absent. As a rule such preparations keep well if protected from moisture, but during the drying they lose much of the flavour of the fresh meat. The best-known examples of this method of preservation are the American preparations, *pemmican*, and *charque*, and *flesh powder*.

Pemmican.—This was formerly prepared by the North American Indians from buffalo flesh, but now usually consists of beef. The flesh is cut into strips, dried in the sun, minced as finely as possible, mixed with equal quantities of fat, and worked up into a paste. According to Dr. Chaumont the finished product contains about 35 per cent. of nitrogenous substance and 56 per cent. of fat.

Charque.—Enormous quantities of this form of dried flesh are prepared in various parts of South America. The meat, after removal of the fat as completely as possible, is cut into thin strips, which are covered with flour, dried in the sun, and rolled and pressed into a compact mass. In Brazil it is mixed with sugar before drying (*charque dulce*), or salted and dried (*carne secca*), or salted and pressed between stones before drying (*carne Tassajo*).

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* *Journ. Pharm. Chim.*, 1892, 25, p. 348.

According to Chevalier * it should have a dark red colour, the muscular fibre should be hard, and no liquid should exude on applying strong pressure with the fingers. Beef loses about a quarter of its weight in the process.

Hofmann † gives the following figures as representative of the composition of fat and lean charque:—

	Per Cent.					Dry Substance.		
	Water.	Nitrogenous Substances.	Fat.	Salts.	Sodium Chloride.	Nitrogenous Substances.	Fat.	Nitrogen.
Fat Charque, .	40·2	48·4	2·1	8·3	6·3	80·93	5·17	12·95
Lean Charque,	36·1	46·0	3·7	15·2	14·1	71·98	4·22	11·91

In 1855 Giradin ‡ made the following comparative analyses of the composition of French beef and South American charque:—

	French Beef.		Charque.	
	Fresh.	Dried at 100° C.	As Imported.	Dried at 100° C.
Water, . . .	75·9	...	49·11	...
Fibrin, . . .	15·70	65·4	24·82	48·78
Fat, . . .	1·01	4·19	0·18	0·35
Albumin, . .	2·25	9·34	0·70	1·38
Extractives, . .	2·06	8·55	3·28	6·44
Soluble Salts, .	2·95	12·24	21·07	41·39
Phosphoric Acid, .	0·222	0·925	0·618	1·216
Nitrogen, . .	3·000	12·578	4·620	9·101
Sodium Chloride, .	0·489	2·030	11·516	22·630

But although *charque* is richer in phosphates and nitrogenous substances than fresh beef, it can only be eaten in small quantities on account of the large amount of salt which it contains, and this also renders it extremely hygroscopic. Moreover, any fat which is left in it is very liable to become rancid. Hence, in spite of its cheapness (25 to 35 centimes per kilo. in La Plata), it has never come into general use in Europe.

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* *Diction. des Altérations et Falsifications*, p. 561.

† *Bedeutung der Fleischnahrung*, p. 162.

‡ *Diction. des Altérations et Falsifications*, p. 562.

Flesh Powder.—Various preparations of powdered flesh or flesh powder have been introduced into commerce, but, as a rule, the difficulty has been to prevent them acquiring an unpleasant flavour from alterations in the fat which they contain. König has confirmed Rubner's statement, that such dried flesh is as digestible as fresh meat.

The lean flesh is dried first on the surface in a special apparatus at a low temperature, which is subsequently raised. When dry the flesh is pulverised and salted.

The following analyses of a German patent flesh powder ('*carne pura*'), which is no longer in the market, have been made by König[*] and Strohmer[†]:—

	Water.	Nitrogenous Substances.	Fat.	N.-free Extractives.	Salts.	Potassium.	Phosphoric Acid.
König, .	10·99	69·50	5·84	0·42	13·25	1·85	1·52
Strohmer, .	10·81	70·24	5·61	...	13·34	...	...

Strohmer also found that 97·56 per cent. of the nitrogenous substance was of a proteid nature, and that 99·2 per cent. of this was digestible. The ash contained 8·77 per cent. of sodium chloride.

Various substances, such as biscuits, meat cocoa, chocolate, etc., have been prepared from such flesh powder. Strohmer gives the following results of the analyses of some of these preparations containing '*carne pura*':—

	Water.	Nitrogenous Substance.	Fat.	Carbohydrates.	Ash.	Digestible Nitrogenous Substances.
Meat biscuit, .	5·98	12·56	12·37	67·09	2·00	92·5
,, cocoa, .	6·25	22·63	30·13	34·65	6·34	65·7
,, chocolate, .	2·10	10·76	25·83	59·10	2·22	72·7

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[*] *Loc. cit.*, ii.

[†] *Die Ernahrung des Menschen*, p. 130.

The writer is indebted to Mr. Otto Hehner for the following analyses of English meat biscuits which are still manufactured:—

	Water.	Fat.	Albumin.	Cellulose.	Ash.	Starch, etc., by difference.
English meat biscuit,	7·53	16·77	16·05	0·97	1·68	57·00

Dried Fish.—In many places small cod, haddock, and stockfish are preserved by slitting them down the middle and drying them in the air. Dried stockfish (*Kabeljau*) is very extensively used. According to Strohmer * it contains:—Water, 16·16; nitrogenous matter, 78·91; fat, 0·78; and salts, 1·52. It is as digestible as flesh powder, and costs less.

Blood Meal.—In Sweden purified, dried blood, in the form of a powder, is a common article of food.*

Preservation by Salting.

This is one of the oldest and most widely used processes of preserving meat. The salt acts partially as a dehydrating agent, combining with the water in the flesh, and partly as an antiseptic, though its value in the latter respect has frequently been overrated.

Methods of Salting.—In one method the flesh is well rubbed with salt, then pressed, the salting repeated, the meat being finally placed in barrels and covered with the salt liquid obtained from the pressings.

Another method consists in placing the meat in casks in layers, with salt between each layer. The salt withdraws water from the flesh, and the brine formed penetrates the fibres.

In Eckart's Munich quick-salting process, the meat is impregnated, under pressure, with a 25 per cent. solution of sodium chloride for twenty-four hours, and then smoked. It is claimed that the loss consists, in the main, of only water and a little phosphoric acid, that the meat has a better flavour, and that any trichinæ are completely destroyed.

Cirio's process, first exhibited in Paris in 1867, is very similar in character, the meat being kept *in vacuo* and brine forced in.

Addition of Nitre.—As one of the results of salting meat is, that decolorisation takes place, it is customary to add a small proportion of potassium nitrate to counteract this. According to Lehmann a very little suffices, but it must not be lost sight of that nitre is a poisonous substance. Five grammes of the salt may cause severe illness, and 8 grammes have been known to cause death. The effect on the human system of the continued use of meat containing nitre has not yet been determined.

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* *Loc. cit.*, p. 132.

Influence of Salting on Bacteria.—From the experiments of Forster * it appears that the streptococci of erysipelas, the bacilli of swine erysipelas, and *Streptococci pyogenes* can live for weeks, and even months, in salted flesh. The bacilli of tuberculosis retain their virulence for over two months, and while the bacteria of anthrax perish in from eighteen to twenty-four hours, their spores retain their vitality for a very long period.

Influence of Salting on the Flesh.—Voit's analysis * tends to show that the nutritive value of flesh is only slightly diminished after fourteen days' salting. He found the percentage loss to be—Water, 10·4; organic matter, 2·1; albumin, 1·1; extractives, 13·5; phosphoric acid, 8·5. The amount of salt taken up by 1000 grammes of the fresh flesh was 43·0 grammes.

E. Polenske,† however, found that beef, after being pickled for three weeks, had lost 7·77 per cent. of its nitrogenous constituents, and 34·72 per cent. of its phosphoric acid. After three months the loss in nitrogen was 10·08 per cent., and the loss of phosphoric acid (P_2O_5) 54·46 per cent., while after six months these figures had risen to 13·78 and 54·60 per cent. respectively.

From this Polenske concluded that the meat was completely altered in character as a nutrient substance. Moreover, on account of the large amount of salt, it cannot be used as a substitute for fresh meat for a continued period without injurious effects.

Strohmer ‡ gives the following comparative analyses of the composition of fresh and salted herring, and of salted anchovies:—

	Water.	Nitrogenous Substances.	Fat.	Ash.	Sodium Chloride.
Fresh Herring,	80·71	10·11	7·11	2·07	...
Salt Herring,	46·2	18·9	16·9	16·4	14·0
Salt Anchovies,	57·8	22·3	2·2	23·7	20·0

The Composition of the Pickling Fluid.—In *Polenske's* experiments this liquid contained nitre and salt, and as the pickling proceeded, the nitrate became reduced to nitrite and ammonia.

Gerlach § gives the following analysis of an old herring pickling fluid:—Water, 74·40; sodium chloride, 22·78; ammonium lactate, 0·68; soluble proteid substances, 0·820; other organic matter, potassium sulphate, and calcium phosphate, 1·352 per cent. Hoffmann[*] and Werthe also found volatile bases, such as trimethylamine and propylamine. The pickling liquid, from whatever flesh originating, is often very poisonous to animals.

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* Ostertag, *loc. cit.*, p. 526.

† *Jahresber. Nahr. u. Genussm.*, 1891, p. 40.

‡ *Loc. cit.*, p. 133.

§ *Handbuch der Fleischkunde*, p. 898.

Caviar.—This is the salted roe of the sturgeon and of other fish. It is prepared by washing the roe with salt water, leaving it in brine for some time, pressing it to remove foreign substances, again treating it with salt water, pressing it through a hair sieve, and finally packing it in salt. In the fresh state caviar is of a greenish shade, which gradually darkens on keeping.

The most prized is the Astrachan caviar, which is prepared at the mouth of the Volga. The German Elbe caviar contains smaller granules, and is prepared from different kinds of fish. It has a sharper taste than the Russian caviar. There is also an American variety, which has small granules, and contains more or less gelatinous matter.

One of the best kinds in commerce is the Saxony caviar, which is packed in linen, and is less salt than the others. The poorer varieties are pressed and salted, and sold as 'pressed caviar' or 'fish-cheese.'

Composition.—From the analyses of Gobley[†] and of König,[‡] caviar has the following proximate composition:—

	Water.	Nitrogenous Substances.	Fat.	N.-free Substances, etc.	Ash.	Dry Substance.		
						N.-substances.	Fat.	Nitrogen.
Caviar, . .	48·13	26·58	14·57	4·16	6·56	...	...	...
,, . .	43·89	30·79	15·66	1·67	8·09	54·89	24·02	8·78
Pressed caviar,	30·89	40·33	18·90	...	9·88	58·36	27·35	9·36

Examination of Caviar.—W. Niebel[§] gives the following as the characteristics of good caviar:—1. The colour should be grey or black; 2. The size of the eggs varies from 2 to 3·5 mm.; 3. There should be no smell, although an acid smell is frequently to be observed in commercial varieties; 4. Foreign substances must be absent, such as hair or sand, due to careless preparation, or oil and sago, fraudulently added.

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[*] *Handbuch der Fleischkunde*, p. 898.

[†] Strohmer, *Die Ernahrung des Menschen*, p. 143.

[‡] *Nahrung Genussmitt.*, ii. p. 128.

[§] *Zeit. Fleisch u. Milch Hyg.*, 1893, i. p. 5.

The best caviar is neutral to litmus paper, but the poorer kinds are usually acid. The latter also frequently contain traces of free ammonia, hydrogen sulphide, and free fatty acids.

Preservation by Smoking.

The process of smoking preserves flesh partly on account of the drying action of the heat and partly through the antiseptic action of some of the substances in the smoke, such as creosote, formaldehyde, and pyroligneous acid. According to Marasse, the creosote in wood smoke consists of a mixture of $C_7H_8O_2$, $C_8H_{10}O_2$ and $C_9H_{12}O_2$. It coagulates the albumin of the meat, forming a protecting envelope. The best wood for the production of the smoke is beech, while pine and fir are quite unsuitable, on account of the resins they contain. There is no loss of nutriment, such as occurs in salting, and Strohmer [*] found that smoked meat was as digestible as fresh meat.

Methods of Smoking.—There are two chief processes of smoking:—1. The flesh is slowly smoked for twenty-four hours at 25° C., or in the case of sausages and fish at 70° C., and then for a short time at 100° C.; or 2. The flesh is placed directly in the hot smoke.

Beu [†] examined a large number of different commercial smoked meat products, and found that those prepared by the slow process contained many more micro-organisms. Intermittent smoking is bad, since it favours decomposition.

Action of Smoke on Bacteria.—Serafini and Ungaro [†] proved that smoke acts very energetically on pure cultivations of bacteria. The bacilli of anthrax and staphylococci perished in two and a half hours at the outside, hay bacilli in three and a half hours, and the spores of the anthrax bacilli after eighteen hours.

On treating flesh infected with anthrax in the same manner, it was found, however, that the bacilli in the interior of the flesh did not perish, since the smoke could only penetrate very slowly on account of the coagulation of the albumin. Hence the conclusion arrived at was that smoking checks the development of bacteria, but does not destroy them.

Forster [‡] met with the same experience in the case of the bacilli of tuberculosis, which he found were still virulent, after the flesh which contained them had been both salted and smoked.

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[*] *Die Ernahrung des Menschen*, p. 135.

[†] Ostertag, *Handb. der Fleischbeschau*, p. 528.

[‡] Ostertag, *loc. cit.*, p. 384.

Composition of Smoked Flesh.—Strohmer* gives the subjoined analyses of various kinds of smoked flesh:—

	Water.	Nitrogenous Substances.	Fat.	Ash.
Ham, ordinary, ...	59·73	25·08	8·11	7·08
,, Westphalian, . . .	27·98	23·97	36·48	10·07
Smoked Beef, ...	47·68	27·10	15·35	10·59
,, Ox Tongue, . . .	35·74	24·31	31·61	8·51
,, Herring, . . .	64·49	21·12	8·51	1·24

The following analyses of smoked and salted meat and fish are taken from König †:—

	Water.	Nitrogenous Substances	Fat.	Ash.	Sodium Chloride.	On Dry Substance.		
						Nitrogenous Substances	Fat.	Nitrogen.
Smoked Horseflesh, .	49·15	31·84	6·49	12·53	...	62·61	12·76	10·02
Westphalian Ham, .	28·11	24·74	36·45	10·54	...	34·41	50·69	5·50
American Bacon, .	9·15	9·72	75·75	5·38	...	10·70	83·38	1·71
,, ,,	10·70	2·62	77·80	6·60	...	2·91	87·12	0·47
Smoked Goose Breast,	41·35	21·45	31·49	4·56	...	36·57	53·69	5·85
Mackerel (mean of 4),	44·45	19·17	22·43	13·82	11·42	34·64	40·10	5·54
Herring (,, 3),	46·23	18·90	16·89	16·41	14·47	35·27	31·20	5·64
Salmon (,, 2),	51·46	24·19	11·86	12·04	10·87	49·88	24·44	7·98

Influence of Smoking on the Flesh.—During the process of pickling and smoking the colouring matter of flesh undergoes alterations, as may be observed with the spectroscope. Utescher ‡ found that smoked ham and Hamburg smoked flesh had invariably an alkaline reaction.

The Composition of American and Dutch Bacon.—Kaiser was unable to find any difference in the composition of these kinds of

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[*] *Die Ernahrung des Menschen.*

[†] *Loc. cit.*, i. pp. 206 and 228.

[‡] *Apoth. Ztg.*, 1894, p. 765.

bacon. Lutz,* however, from the results of his comparative analyses, came to the conclusion that there was a considerable difference, and gave the following figures as representative of their composition:—

	Water.	Nitrogenous Matters.	Fat.	Ash.
American Bacon, ...	9·0	9·0	71·5	10·5
Dutch Bacon, ...	12·0	14·5	63·5	10·0

Preservation by Heat-Sterilisation and Exclusion of Air.

A very large number of processes may be grouped under this head, but all are based to a greater or less extent on that devised by Appert in 1809, in which the provisions were heated in earthenware vessels and protected from subsequent infection by hermetic sealing. In some of the later patents the air in the vessel is replaced by an inert gas such as nitrogen or carbon dioxide, but such methods as these seem unlikely to replace the simpler ones in use.

Canned Meats.—Modifications of Appert's process have been used for the preservation of almost every description of food, but especially for fruit, meat, and fish. Cans are employed to a much greater extent than glass or earthenware, owing to their greater strength, and the readiness with which they can be made air-tight.

In the large American factories, steam retorts are used for the sterilising, but, in the smaller factories, the cans are immersed in boiling water or in a salt bath. A small hole is left in the cover, and, after the sterilisation, and while the can is still filled with steam, this is closed with a fragment of solder. Finally, the cans are left for a week, and are then tested by striking each on the head with a wooden hammer. If the cap sinks down slowly, the process has been properly carried out, but if it is elastic and springs back, it is what is termed a 'swell-head,' and is rejected.

Preparation of Corned Beef.—In the preparation of this, finely-divided flesh, freed from sinews and fat, is pickled in vats, and, when salted, is cooked and packed by means of steam pressure into cans, which are immediately sealed. After standing for three to six hours in boiling water, the cans are pierced to allow water and fat to escape, again soldered up, and again placed in boiling water for several hours.

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* *Jahresber. Nahr. Genussm.*, 1891, p. 40.

Composition of Canned Meats.—König gives the following table of the percentage composition of some well-known canned meats:—

	Water.	Nitrogenous Substances	Fat.	Ash.	On the Dry Substance.		
					Nitrogenous Substances	Fat.	Nitrogen.
American meat, salted,	49·11	28·87	0·18	21·07 (NaCl 11·52)	56·73	0·35	9·08
Prepared by Wilson, .	57·3	28·9	10·2	3·6	67·68	23·75	10·83
„ Canning & Co., .	49·2	25·7	21·6	3·5	50·59	42·52	8·09
„ Brougham,	48·9	27·7	19·0	4·4	54·21	37·18	8·67
Australian, . .	54·03	29·31	12·11	4·55	63·76	26·34	10·20
Pressed corned beef, .	51·9	33·8	6·4	2·9	78·42	14·85	12·55
„ „ .	57·7	31·5	7·3	3·5	74·47	17·62	11·91
„ „ .	58·8	25·9	11·8	3·5	62·86	28·64	10·05
Mean of 10, . .	55·80	29·06	11·54	3·60	66·64	26·10	10·66
Tongue, . . .	64·86	15·35	15·14	2·64	43·64	43·08	6·69
Californian salmon (mean of 3), . .	61·78	20·16	15·68	2·38	53·42	40·36	8·55

Warden and Bose* analysed a number of representative specimens of canned beef and mutton, and compared their results with the figures given by König for fresh meat (*cf.* p. 47).

With seven brands of different manufacturers they obtained the following results:—Water, 49·05 to 57·35; fat, 10·34 to 22·08; nitrogen, 3·93 to 4·65; total ash, 0·62 to 4·36; soluble ash, 0·189 to 4·176; chlorine, 0·112 to 2·65; phosphoric acid (P_2O_5), 0·308 to 0·402; potassium oxide, 0·136 to 0·434; sodium oxide, 0·117 to 0·963; extracted by boiling water, 5·35 to 10·414; and nitrogen in boiling water extract, 0·90 to 1·1 per cent.

The albuminous substances (N × 6·25) in the anhydrous flesh, freed from all visible fat, are compared in a table with those of fresh meat:—

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* *Chemical News*, 1890, 71, pp. 291 and 304.

	Per cent. Albuminous Substances.
Average of Canned Beef Samples, ...	87·06
,, ,, Mutton, ...	87·19
,, Fresh Cow and Ox Flesh, ...	93·94
,, ,, Mutton, ...	93·81
,, all Canned Samples, ...	87·12
,, all Fresh Meat, ...	93·87

From these analyses Warden and Bose came to the conclusion that the nutritive value of canned meat is considerably less than that of fresh meat, this difference being partially due to the salt which was present in large quantity in some of the canned meats.

Sardines in Oil.—In this method of preservation the air is excluded by immersing the fish in olive oil. According to König the fish, from which the oil has been removed by pressing it between folds of filter paper, has the following composition (mean of three analyses):—

Water.	Nitrogenous Substances.	Fat.	Ash.
53·64	25·90	11·27	9·00

On the Dry Substance.		
Nitrogenous Substances.	Fat.	Nitrogen.
36·49	28·01	5·84

Maljean[*] gives the following as the composition of sardines, which had been preserved by Appert's process without the use of oil or sauce:—

Water.	Nitrogenous Substances.	Fat.	Ash.
57·50	28·40	8·07	6·03

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[*] *Rev. internat. des Falsif.*, 1894, p. 133.

Occasionally a red coloration of sardines, preserved in oil, may be observed. This, according to Auché,[*] is due to a chromogenic bacillus, which is found in large numbers on the sardines before preservation. It is distinct from *B. prodigiosus*, and is non-pathogenic.

The Examination of Canned Meats.—On opening the tins no gas should be found, and the jelly surrounding the flesh should be solid. If liquid, it indicates decomposition.

Collection of the Gases.—The method described by Doremus [†] will be found suitable for the collection and examination of the gases in imperfectly sterilised goods.

A hollow, bevelled steel needle is fixed in the upper arm of an adjustable clamp with its point passing through the hole of a rubber cork, which rests upon the top of the can. The upper part of the needle is connected by means of a capillary tube with a gas burette or nitrometer filled with water or mercury. The can, which is held between the lower arm of the clamp and the rubber cork, is then punctured by turning the lower screw until the needle pierces the top. The rubber cork makes a tight joint round the needle, and the gases escape gently into the eudiometer, where they are measured and analysed in the usual way. From 60 to 80 c.c. of gas may sometimes be collected. Doremus states that when there is a putrid odour, carbon dioxide forms the chief constituent of the mixed gases. In other cases hydrogen predominates, there is no offensive smell, and bacteria are absent, whilst there are indications of the corrosion of the inner metallic surface. Hydrogen has also been found without distinctive signs of corrosion. Occasionally the can is discoloured, as though traces of hydrogen sulphide had been formed, and the reactions of the metals may be obtained with the contents of such tins.

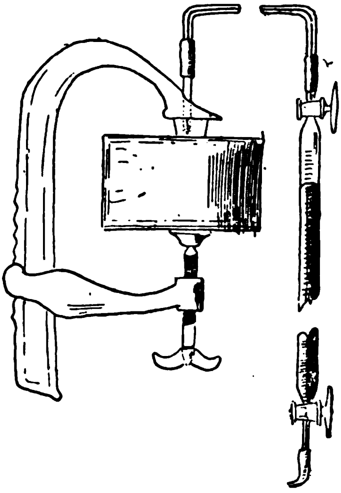

FIG. 18.—Apparatus for collecting gases from cans.

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[*] *Zeit. Fleisch u. Milch Hyg.*, 1894, p. 135.

[†] *Jour. Amer. Chem. Soc.*, 1897, 19, p. 730.

The Chemical Reaction of Canned Flesh.—According to Utescher[*] the flesh preserved in cans often has an alkaline reaction, without the flesh being in any way decomposed. This is noticeably the case with canned lobsters, and the point is one of considerable importance, since the alkaline liquid dissolves some of the metal from the interior of the can. To prevent this certain manufacturers line the interior with a silicate enamel, or with tough parchment paper.

Poisoning by Canned Goods.—From time to time cases of illness are reported, brought on by eating canned food, though such cases are rare, if the enormous quantities of this class of food annually consumed are taken into account. When they do occur they may be due to decomposition of the flesh, through imperfect sterilisation and the consequent formation of ptomaines (*cf.* p. 222), or the flesh used may itself have been poisonous, as is sometimes the case with fresh meat and, more often, with fish (*cf.* pp. 217–220); or it may be due to metal from the interior of the can, dissolved by the liquid present, or from careless soldering.

Metallic Contamination of Canned Goods.—There appears to be little doubt from the work of various chemists that all kinds of canned articles are liable to metallic contamination, the degree depending to a large extent on the length of time the food has remained in contact with the metal. Van Hamel Roos[†] in fact advocates that no tins should be employed for the preservation of food without an interior protective lining of some description.

The different metals are tested for by the ordinary qualitative methods in the ash obtained on calcining portions of the flesh.

Tin.—O. Hehner[‡] examined a large number of tinned animal foods, and discovered tin in almost all. In a 1 lb. tin of soup the quantity was 0·035 gramme, and in preserved oysters (1 lb.) 0·045. In the case of hard meats the metal was found principally on the surface of the food. In some instances the interior of the can was discoloured or blackened, but in others it was still bright, notwithstanding the fact that an appreciable amount of tin had been taken up by the food.

In van Hamel Roos' communication[§] the occurrence and significance of this metal in preserved foods is fully discussed, with references to the results of previous workers. Kayser of Nuremberg has recorded cases of poisoning through eating preserved eels which were subsequently found to contain 0·19 per cent. of tin, and in a case in which 270 soldiers were poisoned by canned lettuces and meat, Bettink of Utrecht found from 0·19 to 0·72 per cent. of tin in the food. In a tin of beef eight years old containing 970 grammes, van Hamel Roos obtained 77 milligrammes of stannic oxide, and all the other articles which he examined were more or less contaminated. In a can of asparagus thirty-one years old, the coating of tin had been completely dissolved off the metal.

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[*] *Apoth. Ztg.*, 1894, p. 765.

[†] Abstract in *Analyst*, 1895, p. 195.

[‡] *Analyst*, 1880, p. 219.

[§] *Apoth. Ztg.*, 1894, p. 765.

Lead.—When this metal is found in canned provisions it is usually derived from the solder of the can. A. Mayer * found in the ash of the meat from three tins of corned beef 0·099 gramme, 0·026 and 0·027 gramme of lead. In the Public Laboratory at Karlsruhe a slice of corned beef 1 cm. thick, weighing 145 grammes, was found to contain 0·09 gramme of metallic particles, whilst 0·01 gramme of lead was found in the ash. Similarly in a tin of ham 0·136 gramme of leaden particles were discovered.

In carefully soldered tins of American manufacture, Gautier could not, however, detect any lead in the food, and the chance of its occurrence is considerably lessened by soldering the tin only from the outside.

Copper.—This may originate from the use of copper vessels in the preparation of the food for canning, or it may have been present as a normal constituent of the food. For instance, Harvey states that in his experience copper is widely disseminated in shell-fish, and that he has always connected the delicate pink colour of the ova of salmon with the presence of a very minute quantity of copper. Hehner, too, found a small amount of copper in tinned oysters (*cf.* p. 68).

Bacteriological Examination of Tinned Meat.—This is carried out by the general methods given on p. 265. A simple microscopical examination is also of considerable importance, and note should be taken whether the muscular fibres still show their cross striations, or whether there is any coloration due to bacteria. If a large number of bacteria are observed it is probable that old or diseased flesh was used, and the presence of poisonous substances (toxalbumoses) is then not improbable.

Potted Meats.—The following results were obtained by König and Söllscher † in the analysis of different varieties of food pastes and potted meats manufactured by Crosse & Blackwell in 1884, with the exception of the *pâté de foie gras*, which was procured from Strassburg:—

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* König, *Die Menschlichen Nahr. u. Genussm.*, ii. p. 155.

† *Loc. cit.*, i. p. 233.

	Water.	Nitrogenous Substances.	Fat.	Nitrogen-free Extractives.	Ash.	Sodium Chloride.
Pâté de foie gras,	46·04	14·59	33·59	2·67	3·11	0·22
Potted beef, .	32·81	17·17	44·63	3·36	2·03	...
Potted ham, .	25·57	16·88	50·88	...	6·78	5·72
Potted tongue, .	41·52	18·46	32·85	0·46	6·71	5·98
Potted salmon, .	37·64	18·48	36·51	0·70	6·67	5·65
Potted lobster, .	51·33	14·87	24·86	4·04	4·90	0·38
Anchovy paste, .	36·81	12·33	1·59	5·18	44·09	40·10

Preservation by Chemical Antiseptics.

Leaving out of the question the antiseptic substances which are employed in smoking and salting flesh preparations (salt, nitre, etc.), the number of chemical agents which have been used as meat preservatives is very large. Fresh meat and fish, potted meat, canned goods, hams, and sausages are often found to have been treated with some antiseptic or other, or with a mixture of several substances, with the object of increasing their keeping qualities.

As to the advisability of this practice various opinions have been brought forward on each side. Although a preservative is a lesser evil than incipient putrefaction, there can be little doubt but that the continued use of food containing such substances has an injurious effect on the consumer. Moreover, in the case of meat, it would seem from Bersch's experiments (*cf.* p. 122) that the treatment of fresh flesh with antiseptics only preserves it superficially, and lulls the purchaser into a false sense of security.

From time to time the results of experiments are published showing that this or that preservative does not interfere with the action of the peptic or pancreatic enzymes in artificial digestion experiments; but such experiments do not show that the secretion of the fluids by the glands in the body is not weakened, or that the absorption of the digested substances by the system is not interfered with. Under the present state of affairs it is possible for a dealer to add what quantity he pleases of these antiseptics, and thus there is considerable reason for the opinion of the majority of the medical men consulted on this subject by the *Lancet*,* that if preservatives are to be allowed in food, it should be made compulsory for the vendor to declare the nature and amount of the compound used.

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* *Lancet*, 1897, pp. 50–60.

Kämmerer analysed twenty-four varieties of meat preservatives, and found they could be classified into four groups, containing—1. Common salt and nitre; 2. Sodium sulphate; 3. Boric acid or borax; and 4. Borax and sodium sulphite.

Among the chemical substances which have been used or recommended for the preservation of flesh are the following:—Sulphur dioxide, various sulphites, bisulphites, sulphates and bisulphates; boric acid, and various borates; fluorides, fluosilicates, fluoborates; chlorides (besides common salt); nitrates of various metals; alum, lime, sodium carbonate, formaldehyde, chloraldehyde, acetic acid, sodium acetate, benzoic acid and benzoates; salicylic acid, sodium salicylate, ethylidene, lactic acid, etc.

Of these compounds, boric acid and borax, salicylic acid and sulphites are the most frequently met with.

Boric Acid and Borax are very widely employed for increasing the keeping properties of hams and fish. According to Jean,[*] borated hams are extensively imported into France from England and America. To preserve fish, boric acid is used in the proportion of 2 grammes per kilo.[†]

Mitscherlich has described the toxic effects of boric acid. It is a cumulative poison, and, according to le Féré, is eliminated from the system but slowly, having been detected in the urine forty or fifty days after it had been taken.[*] Boric acid does not appear to interfere with the process of peptic digestion, so far as can be concluded from artificial experiments.[‡]

There have been numerous cases of flesh poisoning in Switzerland through meat preserved with borates, which have not acted as complete preservatives, but have only masked the incipient putrefaction.[§]

Detection of Boric Acid in Flesh.—Häfelin[||] recommends the following method:—10 grammes of the finely divided flesh (freed from fat as completely as possible) are warmed for about one minute with a mixture of glycerin, 2 c.c.; alcohol, 4 c.c.; and water (just acidified with HCl), 4 c.c.; and the liquid filtered and tested with turmeric paper in the usual manner (brown coloration, turning black on addition of ammonia). As a confirmatory test the residue left on incineration may be moistened with sulphuric acid and methyl alcohol, the flame on ignition having a green tint in the presence of boric acid.

The Determination of Boric Acid in Flesh.—The following method is recommended by C. Fresenius and G. Popp[¶]:—Ten grammes of the finely divided substance are triturated in a mortar with from four to eight times the quantity of calcined sodium sulphate, the mass heated on the water-bath, and, when dry, finely pulverised after the addition of more sodium sulphate. It is then digested in 100 c.c. of cold methyl alcohol for twelve hours, with frequent shaking, and the alcoholic extract distilled. As a rule the whole of the boric acid passes over with the alcohol, but it is advisable to repeat the extraction and distillation, using 50 c.c. of methyl alcohol. The distillates are made up to 150 c.c. with methyl alcohol, and the boric acid determined in 50 c.c. by adding 75 c.c. of water and 25 c.c. of pure glycerin, and titrating with N/20 sodium hydroxide solution, with phenol-phthaleïn as indicator. As soon as a pale rose coloration is obtained more glycerin is added, and if the colour disappears the titration is continued. The number of c.c. of alkali used, multiplied by 0·0031, gives the quantity of boric acid (H_3BO_3) in the liquid titrated. If borates are also present in the substance they should be left in the residue from the distillation, and can usually be extracted with methyl alcohol from the mass after incineration.

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[*] *Rev. de Chim. Ind.*, 1897, p. 289.

[†] Hehner, *Analyst*, 1890, p. 221.

[‡] *Analyst*, 1891, p. 126; Cripps, *ibid.*, 1898, p. 182.

[§] *Jahresber. Nahr. Genussm.*, 1895, p. 76.

[||] *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 188.

[¶] Abstract, *Analyst*, 1897, p. 282.

O. Hehner * mixes the substance with methyl alcohol acidified with sulphuric acid, and collects the boric acid distilling over with the alcohol, in a solution of sodium phosphate of known strength, evaporates the liquid to dryness, and weighs the residue.

K. Thaddéef † recommends a gravimetric method, in which the boric acid distilled over with methyl alcohol is fixed and weighed as potassium borofluoride. The distillate is received in a platinum basin containing a 10 per cent. solution of pure potassium hydroxide, and when four successive portions of 10 c.c. of methyl alcohol have distilled over is concentrated to half its volume on the water-bath. An excess of pure hydrofluoric acid is then added, and the evaporation continued until only a faint smell of hydrofluoric acid is perceptible. When cool, 50 c.c. of a solution of potassium acetate (sp. gr. 1·14) are added, and the basin allowed to stand for one or two hours, its contents being frequently stirred with a platinum rod. The insoluble substance is collected on a weighed filter paper, which has previously been moistened with alcohol and dried at 100° to 110° C. The filter and its contents are washed with alcohol of specific gravity 0·805, of which from 62 to 72 c.c. are usually required, and are then dried at 100° to 110° C. for three hours and weighed.

Sulphur Dioxide and Sulphites.—Sulphurous acid and its salts are very widely employed in the preservation of meat products, and enter into the composition of very many of the meat preservatives with fanciful names now in the market. They all have a powerful germicidal action.

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* *Analyst*, 1891, p. 142.

† *Zeit. anal. Chem.*, 1897, pp. 568–637.

As to their physiological action various statements have been made. Polli [*] found that 8 to 12 grammes of sulphites were not injurious to adults, while others found that children could take 1·8 gramme per day without ill effects. On the other hand, 1 gramme of magnesium sulphite has been found in certain cases injurious to women, causing disorders of the stomach (Bernatzik and Braun).

As an instance of the extent to which flesh preparations are treated with sulphurous acid and sulphites, it may be mentioned that Fischer [†] found that 50 per cent. of the preserved meat products (sausages, etc.) sold in Breslau in 1895 contained sulphites, the quantity of sulphur dioxide in the meat varying between 0·01 and 0·34 per cent.

Action of Sulphurous Acid on Flesh.—Sulphurous acid and its salts, especially calcium bisulphite, appear to have a considerable action on muscular fibre, altering the normal condition of the flesh. According to A. Riche, [‡] this action proceeds at the ordinary temperature, and causes changes in the soluble proteid substances.

An addition of 1 per cent. of a sulphite to the flesh is not perceptible either to the taste or smell. On cooking the flesh the sulphite is only partially decomposed and expelled.

Detection of Sulphurous Acid.—To detect sulphur dioxide in flesh H. Kämmerer [§] places a sample on a moist strip of potassium iodate starch paper, and moistens the flesh with sulphuric acid (free from oxides of nitrogen). In the presence of sulphites a pronounced blue colour is immediately obtained, whilst pure flesh only gives at most a feeble coloration after a considerable time. Salted flesh or flesh containing nitre cannot be tested in this way, since by the action of the sulphuric acid substances are set free (HCl, and nitrous acid from nitrites present in the nitre), which liberate iodine from the iodate. In many cases it is possible to recognise the smell of sulphur dioxide on simply mixing the flesh with dilute sulphuric acid (1 : 8). Kämmerer found in the mean 0·0512 gramme of sulphur dioxide, and 0·1016 gramme of sodium sulphite in 100 grammes of preserved flesh.

Estimation of Sulphurous Acid in Flesh. [‖]—A weighed quantity of the finely divided flesh is mixed with phosphoric acid, and distilled in a current of steam or carbon dioxide, the distillate being collected in an apparatus containing an excess of iodine solution. After the distillation the sulphuric acid in the distillate is precipitated with barium chloride in the usual way. According to Fischer, all flesh containing more than 0·1 per cent. of sulphur dioxide must be regarded as injurious to health.

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[*] Ostertag, *Handbuch der Fleischbeschau*, p. 580.

[†] *Forschungs Ber.*, 1897, p. 26.

[‡] *Journ. Pharm. Chim.*, 1897.

[§] *Forschungs Ber.*, 1896, p. 257.

[‖] B. Fischer, *Jahresber. Nahr. u. Genussm.*, 1895, p. 76.

Salicylic Acid is one of the constituents of many of the so-called 'meat-preservatives,' although in the case of fresh meat, at any rate, its action appears to be purely superficial. Bersch [*] placed a portion of the flesh of a recently-killed animal in a concentrated aqueous solution of salicylic acid, and found that after four days the exterior of the meat was perfectly sound, whilst the interior showed unmistakable signs of putrefaction, and contained a large number of micro-organisms. Hence he came to the conclusion that the preservation of fresh raw meat by salicylic acid and other ordinary chemical antiseptics was not practicable. In flesh preparations such as sausages and potted meat, in which the salicylic acid can be distributed throughout the mass, its germicidal properties would obviously be more distributed. But on account of its marked taste it cannot be used in such quantity in meat preparations as in some other food substances in which the flavour is concealed.

With regard to the influence of salicylic acid on the human subject there are diverse opinions, but it is significant that the Paris Academy of Science forbid even the smallest addition of salicylates to food as being liable to cause injury where any weakness of the kidneys or digestive organs exists.

Detection and Estimation of Salicylic Acid in Flesh.—The finely-divided flesh is distilled with steam, and the last portions of the distillate tested with ferric chloride (violet coloration). For the estimation the substance is dried, finely pulverised, mixed into a paste with dilute sulphuric acid and extracted with ether. The ethereal extract is evaporated to dryness, and the residue taken up with water and distilled. The free salicylic acid in the distillate is determined by titration with standard alkali, either litmus or phenol-phthalëin being used as an indicator.

The violet coloration given with iron salts may also be employed for the colorimetrical estimation of salicylic acid in the final distillate, the colour obtained on the gradual addition of a very dilute solution of ferric chloride being compared with that given by an aqueous solution of a known quantity of the acid.

An additional test for salicylic acid is to warm portions of the meat product with methyl alcohol and sulphuric acid, when, in the presence of the antiseptic, the characteristic odour of methyl salicylate will be observed.

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[*] *Die Conservirungsmittel*, p. 86.

Formaldehyde.—During the last few years this powerful antiseptic has been tried for the preservation of every description of food, and although its application has not been very successful in the case of flesh, it is still met with in various meat preservatives. 'Carnolin,' for instance, consists of a 1·5 per cent. aqueous solution of formaldehyde slightly acidified.

The effects of the continued use of 'formalin' on the human system have not yet been clearly determined, but its power of forming insoluble compounds with proteid bodies, and its hardening influence on animal tissues, must of necessity render meat treated by it much less digestible if not altogether uneatable. Mabery and Goldsmith [*] found that 0·2 gramme of formaldehyde interfered with the artificial peptic digestion of blood fibrin.

Effect on Meat and Fish.—E. Ludwig [†] states that formalin is not applicable to the preservation of meat products. Ehrlich [‡] tried the effect of an 8 per cent. solution of formaldehyde on various food substances. He found that horse-flesh was completely preserved by it, but that the odour developed was so unpleasant that the meat could not be eaten. Beef treated with the solution was equally preserved, and did not develop this characteristic smell, but, on the other hand, the meat was only fit to be eaten for a short time after the addition of the preservative, on account of the chemical changes caused by it. According to Bloxam,[§] fish treated with formaldehyde becomes so hard as to be unsaleable, even when the preserving solution only contains 1 part in 5000.

The Detection of Formaldehyde. — The finely-divided meat product is mixed with water and distilled, and the distillate tested for the preservative. A very large number of tests have been described, of which the following are a selection:—

1. A drop of milk is added to the distillate, and the mixture poured carefully down the side of a test-tube containing strong sulphuric acid, with a trace of ferric chloride. A blue ring appears at the zone of contact of the liquids in the presence of traces of formaldehyde (but only with traces). Acetaldehyde does not give this reaction (Hehner).[‖]
2. One drop of a dilute aqueous solution of phenol is added to the distillate, and the mixture added to strong sulphuric acid, a bright crimson ring appearing at the line of contact, in the presence of formaldehyde. This coloration is perceptible in solutions containing 1 part

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[*] *Jour. Amer. Chem. Soc.*, 1897, p. 889.

[†] *Zeit. Fleisch u. Milch Hyg.*, 1894, p. 193.

[‡] *Ibid.*, 1898, p. 232.

[§] *Analyst*, 1895, p. 167.

[‖] *Ibid.*, 1896, p. 96.

of formaldehyde in 200,000. If more than 1 part in 100,000 be present, a white milky zone appears above the red ring, while in still stronger solutions a pink curd-like precipitate is obtained. Acetaldehyde, under the same conditions, gives an orange-yellow coloration (Hehner).*

3. Nessler's reagent mixed with solutions of formaldehyde gives a brown coloration, or precipitate, which gradually darkens and finally becomes dark grey. This reaction, which is not given by acetaldehyde, will detect a very minute trace of formaldehyde (Mitchell).†
4. The distillate floated on an equal volume of a solution of 0·1 gramme of morphine hydrochloride gives a reddish violet colour in a few minutes, if formalin be present in greater quantity than 1 : 6000 (Kentmann).‡
5. Several drops of a 10 per cent. solution of phloroglucinol are added to 10 c.c. of the distillate, the mixture shaken, and a few drops of a solution of potassium hydroxide added. A red colour is obtained in a solution containing as little as 1 part of formaldehyde in 20,000 (Jorissen).§

The Estimation of Formaldehyde.—Owing to its power of combining with proteid substances to form insoluble non-volatile compounds, it is practically impossible to determine the exact amount of formalin added to a meat product, and the amount obtainable by distillation decreases with the lapse of time.

One of the simplest methods of estimating formaldehyde in an aqueous solution is based on the fact that it combines with ammonia to form hexa-methylene-amine—

$$6CH_2O + 4NH_3 = (CH_2)_6N_4 + 6H_2O.$$

A known volume of the solution is shaken in a stoppered bottle with an excess of standard ammonia, the bottle allowed to stand for several hours, the uncombined ammonia distilled over into standard acid, and the latter titrated back. A correction is made for the acidity of the solution previously determined by titration with standard alkali.

H. Smith describes a method of determining formaldehyde by oxidation with potassium permanganate,|| and Romijn discusses the accuracy of the various methods of estimation, and describes two new processes.¶ See also a paper by R. Orchard (*Analyst*, 1897, p. 4).

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* *Analyst*, 1896, p. 96.

† *Ibid.*, 1896, p. 98.

‡ *Ibid.*, 1897, p. 106.

§ *Ibid.*, 1897, p. 282.

|| *Ibid.*, 1896, p. 148 ; 1897, p. 5.

¶ *Ibid.*, 1897, p. 221.

CHAPTER VII.

THE COMPOSITION AND ANALYSIS OF SAUSAGES.

In this country only two or three kinds of sausages are manufactured, but on the Continent, and especially in Germany, where the sausage may be regarded as a national dish, there are many varieties prepared by different recipes.

German Sausages.—The chief kinds of German sausages, as described by König [*] and by Merges,[†] are:—

Red Sausage (*Rothwurst*, *Buntwurst*).—Pork is boiled for about three-quarters of an hour, flavoured with salt, pepper, pimento, etc., and after admixture of not too great a proportion of warm fresh blood, placed in the skins and boiled. The English *black puddings* have a similar composition.

Magenwurst.—This is very similar in composition to *rothwurst*, but contains less blood and rather more fat. The sausage mass is finally packed in a cleansed pig's stomach.

Zungenwurst is composed of tripe, pig's head, and fat pork from a young pig, finely minced and mixed with a small quantity of pig's liver and some pig's blood.

Blutwurst is composed of bacon and pork, sometimes with the addition of heart and kidney, and with or without flour. It is mixed with about an eighth of its weight of fresh pig's blood, and boiled.

Lungenblutwurst differs from the preceding sausage in containing finely minced lung.

Leberwürste, or *Liver Sausages*, are prepared from pigs' or calves' livers, thoroughly cleansed from blood, and mixed with a certain proportion of lard and pork, and cooked. The constituents and the proportions vary in the different kinds of liver sausage, such as Mecklenburg leberwurst and Brunswick leberwurst.

Gehirn, or *Brain Sausage*, consists principally of calves' brains and pork.

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[*] *Die Menschlichen Nahr. u. Genussm.*, ii. p. 161.

[†] *Wurst und Fleischwaaren Fabrikation.*

Presskopfwurst is largely composed of pickled and boiled pig's head.

Mosaik Sausage is a mixture of pork and beef, with spices, etc.

Abfallblutwurst is made from sinews and butchers' refuse, with bacon and pig's or ox blood.

Schwartenwurst and *Sulzenwurst* consist of lightly cooked unsalted ham, together with skin, etc., boiled soft, and a little blood.

Bratwurst is prepared from fresh raw pork and ham, with salt, pepper, etc., and sometimes contains lemon-peel or cumin.

Cervelatwurst is prepared from pork and lard, often with the addition of beef or horse-flesh. This sausage is often coloured with fuchsin.

The Italian *Salamiwurst* is manufactured from beef or pork, and is coloured with red wine.

Knackwurst, or *cracking-Sausage*, is a hard, smoked sausage, about 15 cm. in length, with the same composition as *cervelatwurst*, but differing in the flesh being previously cooked. Its name is derived from the crackling sound on breaking the sausages apart.

Knoblauchwurst, or *Garlic Sausage*, has the same composition as the preceding sausage, with the addition of garlic.

Frankfort or *Vienna Würstchen* are small sausages about the length of a finger, composed of raw, lean pork, seasoned with salt, pepper, etc.

Erbswurst consists of a mixture of beef-fat, bacon, pea-meal, onions, salt, and seasoning. The pea-meal is prepared by a patent process to prevent the development of acidity. These sausages formed a principal part of the rations of the German troops in the Franco-German war.

The Table on p. 127, taken from König's larger list, gives the composition of some of these varieties of sausages.

English Sausages.—The best kinds of sausages sold in this country are prepared from raw meat, suitably flavoured with spices, and frequently incorporated with a small proportion of bread-crumbs. In poorer districts, however, the amount of bread or powdered biscuit often exceeds that of the meat. A remarkable instance of the extent to which this practice has been carried on was revealed in a recent case in the law courts, in which a baker admitted that the sausages from which he made his sausage-rolls contained no meat at all, but were prepared from bread coloured with red ochre, and seasoned.

The innumerable varieties of sausages met with in Germany are not manufactured in England, where sausages are usually described by the name of the meat they contain.

COMPOSITION OF GERMAN SAUSAGES.

	Water.	Nitrogenous Substances.	Fat.	Carbo-hydrates.	Ash.	On the Dry Substance.		
						Nitrogenous Substances.	Fat.	Nitrogen.
Cervelat Sausage, ...	37·37	17·64	39·76	...	5·44	28·17	63·47	4·35
Mettwurst (Westphalian), . .	20·76	27·31	39·77	5·10	6·95	34·59	50·33	5·51
Franfurter Würstchen, . .	42·79	11·69	39·61	2·25	3·66	20·43	69·24	3·27
Blutwurst (best quality), . .	49·93	11·81	11·48	25·09	1·69	23·59	22·90	3·77
,, (ordinary), . .	63·61	9·93	8·87	15·83	1·76	27·29	24·37	4·37
Leberwurst, I. quality, . .	48·70	15·93	26·33	6·38	2·66	31·05	51·33	4·97
,, III. ,, . .	47·58	10·87	14·43	20·71	2·87	20·74	27·52	3·32
,, ordinary commercial,	55·73	9·09	14·76	19·33	1·09	20·53	33·34	3·29
Sülzenwurst, ...	41·50	23·10	22·80	...	12·60	39·49	38·96	6·31
Knackwurst, ...	58·60	22·80	11·40	...	7·20	55·07	27·53	8·81
Erbswurst (German), . .	6·53	15·46	37·94	31·38	8·69	16·54	42·01	2·65
Trüffelwürst, ...	43·29	13·06	41·27	...	2·41	23·03	72·77	3·68
Ham Sausage, ...	46·87	12·87	24·43	12·52	3·31	24·22	45·93	3·88

The following results were obtained by the author in the analysis of typical English sausages, costing 10d. and 8d. per lb. respectively; but obviously the amount of the different constituents may vary widely from these figures:—

	Water.	Fat.	Nitrogenous Substances, N × 6·25.	Starch.	Ash.
Pork Sausages, .	51·20	26·53	11·42	4·20	3·84
Beef Sausages, .	48·64	24·68	10·45	10·52	4·06

Black-puddings are the English equivalent of the German *Blutwurst.* They contain a large proportion of blood, and undergo decomposition with more readiness than ordinary sausages.

Polony Sausages derive their name from a corruption of 'Bologna,' a town celebrated for its sausages. They contain partially cooked pork. A. H. Allen * gives the following analysis of a sample of these sausages:—Water, 45·57; fat, 32·66; proteids, 17·26; gristle, etc., 0·54; starch, 2·30; and ash, 2·80 per cent.

Saveloys are short, thick sausages, which owe their name to the fact of having originally contained brains (French, '*Cervelet*'). At the present time they usually consist of highly-seasoned salt pork.

French Sausages.—The chief difference in the manufacture of French and English sausages is the enormous extent to which horse-flesh is admittedly used (*cf.* p. 56). In their general composition they closely resemble varieties found in England and Germany.

Saucisses consist of a skin of pig's intestine filled with raw or smoked minced flesh (usually pork), and seasoned with salt, pepper, pimento, etc. They are termed *saucisses longues* or *saucisses plates,* according to their form.

Saucissons only differ from *saucisses* in being larger, more compact, and generally more highly seasoned. There are many varieties, such as *Saucissons de Lyons, saucissons cru,* etc.

Cervelas are large, short sausages containing salted and spiced flesh. They appear to be analogous to the English *saveloys.*

According to L. Baillet,† French sausages are not intended to be kept for more than a few days. While still firm to the touch and sound, they gradually acquire on keeping a sharp but not unpleasant flavour, and are then termed *piqué* by the manufacturers. At a more advanced stage of alteration the exterior assumes an earthy tint, and is sometimes perceptibly moist to the hand, these changes being accompanied by the production of an acid and disagreeable odour. This condition is termed *échauffé*. Baillet states that in the east of France, the addition of starch (up to 15 per cent.) is a common practice.

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* *Commercial Organic Analysis,* iv. p. 280.

† *Traité de l'Inspection des Viandes,* p. 466.

The Water in Sausages.—The drier a sausage the better its keeping properties, but according to Serafini,[*] the most suitable proportion of water is from 35 to 40 per cent.

The quantity of water which can be introduced into a sausage is a matter of considerable importance. Trillich found that it was possible to add as much as 70 per cent. without rendering the sausage unsaleable. The absorption capacity of the muscular fibre varies in the case of the flesh of different animals. Thus it is greater in animals which have been regularly fed than in those which are rapidly fattened. According to Ostertag,[†] the proportion of water which beef is capable of absorbing can be artificially increased by working up the flesh before the animal heat has left it. Pork, which has a poor absorptive capacity, can be improved by being salted with frequent turning, and also by the addition of beef or veal. It is usual to add a certain proportion of flour or starch to German sausages to increase their power of absorption, or their cohesive capacity. According to Lintner, starch can absorb 40 per cent. of water without appearing wet.

H. Trillich,[‡] however, finds that an addition of 5 per cent. of starch has no influence on the proportion of water which the sausage can retain. He gives the following description of the method of preparing Munich sausages:—A mixture of veal and pork is finely minced, and from 3 to 5 per cent. of salt and spices added. This mass, which contains about 64 per cent. of water, is the *brat*. From 1 to 10 per cent. of starch is then added, together with sufficient water to give the mass the consistency of the starch-free *brat*. The sausage is placed for twenty to twenty-five minutes in water at 70° C., and smoked for one to one and a half hours.

To determine the proportion of water to *brat*, Trillich[§] takes the water in the original *brat* at 60 to 64 per cent. Assuming it to be 60 per cent., he finds the subsequent percentage addition of water in the sausage by the equation

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[*] *Jahresber. Nahr. Genussm.*, 1892, p. 44.

[†] *Loc. cit.*, p. 505.

[‡] *Report of the Sixth Assembly of Bavarian Chemists*, 1887, p. 95.

[§] *Zeit. angew. Chem.*, 1888, p. 492.

$$\phi = a - 1{\cdot}5\,(100 - a - s),$$

or in percentage of the starch-free *brat*, by

$$\zeta = \frac{100[(100 - s) - 2{\cdot}5(100 - a - s)]}{2{\cdot}5(100 - a - s)},$$

in which a represents the water actually determined in the sausage and s the amount of starch.

If 64 be taken as the percentage of water in the *brat*, the factor 1·5 becomes $1{\cdot}78 = \frac{64}{38}$ in the first equation, and 2·5 becomes 2·78 in the second equation.

The Specific Gravity.—Kämmerer,* finding that the specific gravity of sausages rose or fell according to the amount of water, attempted to approximately estimate this constituent by determining the specific gravity. In practice, however, he found this impracticable, since the space left on expelling some of the water during the smoking and drying became partially filled with air, which interfered with the accurate determination of the density. He gives the following figures as representative specific gravities:—Pork, 1·0611; beef, 1·0731; liver and blood sausages, 1·0350–1·0373; Frankfort liver sausage, 1·0266.

The Determination of Flour or Starch in Sausages.—In some countries an addition of starch to sausages is altogether forbidden. Thus, in Austria, it is only permissible to add a small quantity to Augsburg sausages, while there must be no addition to other meat sausages. In this country, as there is no regulation on the subject, the cheaper kinds of sausages are frequently composed of a large percentage of bread; and in a recent police-court case evidence was given by an apprentice to the effect that he had been taught how to make sausages entirely of bread crumbs, coloured and flavoured to imitate meat.

Qualitative Test for Starch.—A thin section of the sausage is tested under the microscope with a drop of iodine solution, or a small portion of the sausage mass is triturated with water, and a drop of the liquid tested.

Quantitative Methods of Estimating Starch.—i. *Indirect Estimation.*—An approximate idea of the amount of starchy substance may be formed by deducting from the original substance the amount of water + nitrogen multiplied by 6·25 + fat + crude fibre

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* *Report of the Sixth Assembly of Bavarian Chemists*, 1887.

+ mineral matter. The difference gives approximately the N.-free extract.

ii. *Inversion with Malt Extract or Diastase.*—Medicus and Schwab[*] use a malt infusion prepared by digesting 5 grammes of malt with 50 c.c. of water for one and a half hours at 20° to 30° C. Twenty grammes of the sausage material are mixed with 20 c.c. of the malt infusion, the liquid made up to 100 c.c., and left for two hours at 40°–50° C., and then for eighteen hours at the ordinary temperature. The liquid is then filtered, the residue well washed, the filtrate boiled, and the coagulated albumin filtered off. The dextrins present are inverted by heating the liquid with hydrochloric acid, and the dextrose determined gravimetrically or volumetrically with Fehling's solution, a deduction being made for the sugar in the malt extract used.

C. Amthor[†] heats a weighed quantity of the sausage with 95 c.c. of a solution of diastase and 5 c.c. of hydrochloric acid (sp. gr. 1·124) in a stoppered bottle, immersed for three hours in a hot brine bath. The solution is made up to a definite volume, and the sugar determined with Fehling's solution. He makes an allowance of about 1 per cent. for the starch in the pepper and other spices.

iii. *Inversion of the Starch by Acids.*—According to H. Frickkinger[‡] dilute hydrochloric acid does not invert the whole of the starch in sausages, and he therefore recommends digesting the substance on the water-bath with sulphuric acid (5 per cent.) until no precipitate is formed on the addition of alcohol to the filtered liquid.

König[§] extracts 5 to 10 grammes of sausage with boiling absolute alcohol and ether to remove the fat. The residue is heated in a Reischauer pressure flask for four hours in a glycerin bath (130°–140° C.), and when cooled to 90° C. the undissolved matter is separated by filtration and washed. The filtrate is made up to 200 or 250 c.c., and digested for three hours with 10 to 20 c.c. of hydrochloric acid. After nearly neutralising the liquid with potassium hydroxide the sugar in an aliquot portion is determined with Fehling's solution.

From the recent experiments of Sherman[||] the method of extracting the starch with malt infusion or diastase solution must be regarded as the most accurate for the determination of that substance in cereals.

iv. *Digestion with Potassium Hydroxide.*—J. Mayrhofer[¶] considers the following method more simple and reliable than the inversion processes described above. From 60 to 80 grammes of the sausage are heated on the water-bath with alcoholic potassium hydroxide (8 per cent.), which, in the case of pure sausages, dissolves almost everything except a little cellulose. In order to prevent gelatinisation the solution is diluted with warm alcohol, and filtered through a paper or asbestos filter. The insoluble residue, containing the starch when present, is washed with water until the washings are no longer alkaline, then treated with *aqueous* potassium hydroxide, and the starch solution made up to a definite volume. On adding alcohol to an aliquot portion the starch falls as a flocculent precipitate, which rapidly subsides. This is collected on a weighed filter, washed with alcohol and ether, dried and weighed.

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[*] *Berichte d. d. Chem. Gesell.*, xii. 1285. [†] *Rep. f. anal. Chem.*, 1882, p. 356.
[‡] *Zeit. anal. Chem.*, 1880, p. 493. [§] *Loc. cit.*, ii. p. 167.
[||] Abst. *Analyst*, 1897, p. 19; *cf. ibid.*, 1898, p. 218.
[¶] *Zeit. Nahr. Untersuch.*, 1896, p. 331; Abst. *Analyst*, 1897, p. 11.

In order to avoid a determination of the ash it is advisable to make the starch precipitation from a weak acetic acid solution instead of from an alkaline solution, since the acetate formed from the potassium carbonate present is readily soluble in alcohol. By this means the starch is obtained quite free from ash.

Mayrhofer gives the results of analyses of known mixtures of sausage with pure potato starch, which show that this method is very accurate.

v. *Colorimetrical Determination.*—G. Ambühl[*] has devised a colorimetric process, which consists of extracting the starch from the sausage with water, as in König's method (p. 131), and comparing the colour produced on the addition of iodine with that given by a solution containing a known quantity of starch under the same conditions. As a test of this method he prepared sausages containing 1·57 of wheat flour, and in two determinations found 1·4 and 1·5 per cent.

For a discussion of the applicability of determining starch colorimetrically, see Littleton, Abst. in the *Analyst*, 1897, p. 73.

Determination of the Acidity.—A weighed quantity of the finely divided sausage (25 grammes) is digested three times with successive portions of boiling water under a reflux condenser, the aqueous extracts filtered, and the residue washed. The total filtrate and washings are titrated with decinormal alkali, and the acidity calculated as lactic acid.

Kämmerer[†] obtained the following results by this method :—

	Alkali.	Lactic Acid.
	c.c.	per cent.
Raw Sausage (smoked), ...	8·0	0·720
" "	9·5	0·855
Smoked Beef, ...	8·0	0·720
Fresh Pork, ...	4·0	0·360

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[*] *Chem. Zeit.*, 1895, p. 805.

[†] *Report of the Sixth Assembly of Bavarian Chemists.*

As several micro-organisms are able to produce lactic acid, this gives an additional means of judging as to the freshness of meat preparations, especially when considered in conjunction with the results of a determination of the acidity of the fat (rancidity). A distillation of the volatile acids may also enable one to decide whether the flesh has been smoked, since pyroligneous acid is one of the constituents of wood smoke.

The Acid Value of the Fat.—This is determined by the method given on p. 95, and, when the fat is rancid, serves to some extent as an indication of the degree of rancidity. M. Mansfield[*] made experiments to determine whether the fat towards the exterior of the sausage was more rancid than that in the interior. In one Salami sausage the fat near the outside had an acid value of 34, whilst that of the interior fat was 38. In another the fat throughout had an acidity of 43.

The Determination of Gristle in Sausages.—An approximate estimation of the amount of gristle and similar substances may be made by the method described by A. H. Allen.[†] Twenty grammes of the sausage are disintegrated in cold water and the fragments of gristle removed with forceps (by the aid of a lens), washed with methylated spirit and ether, dried at 100° C., and weighed. The nitrogen which they contain is then determined by Kjeldahl's process, and deducted from the total nitrogen originally found in the sausage. The difference (taken as proteid nitrogen) multiplied by 6·3 gives some idea of the amount of gelatinoid substances.

By this method Allen found the following percentages of gristle in different kinds of English sausages:—Pork, 0·67; 'Cambridge' pork, 0·72; Mutton, 3·11; German, 1·13; Polony, 0·54.[†]

The Detection of Horseflesh in Sausages.—Reference was made in a preceding chapter (p. 56) to the fact that a large number of the horses slaughtered in Paris are made into sausages, the vendors of which are supposed to declare that horseflesh is present. In Germany and Austria horseflesh is also largely eaten, and a considerable proportion of it is used up in sausages, either openly or surreptitiously. In England the prejudice against the use of horseflesh, the heavy penalty for selling it without a prominent notification of its nature, and the fact that there is a ready sale on the Continent for exported broken-down horses, all tend to render its occurrence in English sausages unusual.

During the last few years the problem of detecting horseflesh has come into prominent notice, and several methods have been described, and confirmed or modified by subsequent workers.

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[*] *Zeit. Nahr. Hyg.*, 1893, p. 393.

[†] *Commercial Organic Analysis*, iv. p. 281.

i. *Treatment of the Flesh Fibres with Acetic Acid and with Alcoholic Potassium Hydroxide.*—Stelzer based a method of detecting horseflesh on the changes in colour which the muscular fibres of different kinds of flesh undergo on treatment with these reagents. Ostertag,* however, found that there was no marked difference between the colours thus obtained with horseflesh and with beef (especially bull's beef), although it was possible to distinguish them both from pork. On treating the flesh with alcoholic potassium hydroxide (20 grammes of KOH in 100 c.c. of 70 per cent. alcohol), the muscular fibres of beef and horseflesh turn brown, while pork fibres only become white or grey. But, since horseflesh is only employed fraudulently as a substitute for beef, the test is only of positive value in proving that a sausage consists of pork only.

ii. *Treatment with Formaldehyde.*—According to E. Ehrlich,† horseflesh, on treatment with formaldehyde, develops an intense characteristic smell within forty-eight hours resembling that of roast goose flesh. Only in one instance has a faint suspicion of this odour been observed in the case of beef, and Ehrlich considers that this difference may give a further means of distinguishing between the two kinds of flesh.

iii. *The Glycogen Reaction.*—The occurrence of glycogen in appreciable quantities in horseflesh had often been noted, but it was not until 1891 that Niebel made use of its quantitative determination as a means of distinguishing horseflesh from other kinds of flesh (*vide infra*, p. 136).

Two years later Bräutigam and Edelmann ‡ based a method on the well-known colour reaction which glycogen gives with iodine:—Fifty grammes of the finely-divided flesh are boiled for an hour with four times the volume of water, and dilute nitric acid added to the resulting broth, when cold, with the object of precipitating proteid substances and decolorising the liquid. The filtrate is tested with a freshly-prepared saturated aqueous solution of iodine, which is added so as to form a layer on the surface of the liquid. In the presence of glycogen a wine-red ring is formed at the point of contact. When the colour does not appear or is uncertain, the flesh is heated on the water-bath with a solution of potassium hydroxide (3 per cent. of KOH calculated on the flesh) until the muscular fibre is decomposed. The broth is concentrated to half its volume, the proteid precipitated with nitric acid, and the iodine solution added as before.

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* *Zeit. Fleisch u. Milch Hyg.*, 1895, p. 184.

† *Ibid.*, 1898, p. 232.

‡ *Pharm. Centralb.*, 1893, p. 557.

Bräutigam and Edelmann only obtained this reaction with horseflesh, and with the flesh of the human fœtus and the fœtus of animals, but never in their numerous experiments with the flesh of the ox, calf, sheep, pig, dog, or cat. They found that they could detect as little as 5 per cent. of horseflesh in a mixture, and also that it was possible to employ the reaction for an approximate colorimetrical estimation.

This method was tested by M. Humbert,* who examined various kinds of flesh. Of ten specimens of horseflesh obtained from different dealers in Paris, seven showed the colour very clearly; in two it was less pronounced, but still clear; while in the last it was doubtful. In no case was there any coloration with beef, veal, mutton, or pork. Beef-broth left in contact with the iodine for ten days showed no signs of change. The flesh of the ass also gave a negative result, but with that of the mule the reaction was the same as with horseflesh. A mixture of equal parts of horseflesh, beef, veal, mutton, and pork showed the coloration, but it was less pronounced than with horseflesh alone.

The results obtained by W. Niebel † were not so favourable. This chemist considers Bräutigam and Edelmann's reaction uncertain, on the ground that glycogen also occurs in the flesh of dogs, cats, and very young calves; in the livers of cattle, and in meat extract to the amount of 1·5 per cent. In old sausages composed of horseflesh the reaction for glycogen was always obtained, although that substance would usually be decomposed under such circumstances. A further uncertainty is the fact that dextrins derived from the starch give a similar coloration. He maintains that the red colour obtained with iodine is not sufficient proof of the presence of glycogen, which should be isolated in a pure condition. Nevertheless, in his opinion, the iodine coloration, and the occurrence of more than one per cent. of grape sugar in the fat-free substance, point to the presence of horseflesh, even when all the glycogen has been decomposed. In his experience the red colour only fails in the case of the flesh of young foals.

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* *Journ. Pharm. Chim.*, 1895, p. 195. † *Zeit. Fleisch u. Milch Hyg.*, 1895, p. 86.

Drechsler came to the same conclusion as Niebel, since in his experiments on the glycogen test he obtained a wine-red coloration with ten specimens of beef.

The following modified process was devised by Courlay and Coremons.* About 50 grammes of the finely-minced flesh are boiled for fifteen to thirty minutes with 200 c.c. of water. After cooling, the broth is filtered, and tested with a few drops of iodine solution prepared by dissolving two parts of iodine and four parts of potassium iodide in one hundred parts of water. A brown coloration, disappearing on warming to 80° C., and reappearing on cooling, indicates the presence of horseflesh. When flour or starch is present, as in sausages, the blue colour may mask the glycogen reaction. This is obviated by adding two or three times the volume of concentrated acetic acid to the broth, filtering, and again testing the filtrate with the iodine solution. No reaction was obtained in this way with the flesh of cattle, calves, pigs, dogs, or cats, but this observation did not apply to the flesh of the fœtus of any of these animals.

T. Bastien † has recently examined these various modifications, and has found both the original process of Bräutigam and Edelmann, and the preceding modification inconclusive. He has made a long series of experiments under varying conditions, and finds that the following slight modification gives the most satisfactory result, and is capable of detecting 5 per cent. of horseflesh, even in the presence of starch. About 20 grammes of the finely divided sausage are boiled for thirty minutes to one hour with 100 c.c. of water, so that the volume of the liquid is reduced to about 30 c.c. When cold the broth is filtered and about 10 c.c. tested with two or three drops of iodine water, or of a solution of iodine, 1 gramme; potassium iodide, 2 grammes; water, 100 c.c. A fugitive reddish-violet *colour* is obtained with horseflesh. Care must be taken not to add an excess of the iodine reagent, or the colour will change to reddish-brown. When starch is present, acetic acid is added as in Courlay and Coremon's modification.

iv. *The Quantitative Determination of Glycogen.*—In Niebel's ‡ method the flesh is heated on the water-bath for six or eight hours with from 3 to 4 per cent. of potassium hydroxide, and four times its volume of water. The broth thus obtained is evaporated to half its bulk, and hydrochloric acid and a solution of mercuric iodide in potassium iodide (Brücke's reagent), added to the cold liquid, to precipitate nitrogenous substances. The clear filtrate is mixed with two and a half times its volume of 90 per cent. alcohol, and the precipitated glycogen collected on a filter, washed successively with 60 per cent., 90 per cent., and absolute alcohol, with ether, and again with absolute alcohol, dried at 110° C., and weighed.

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* *Zeit. Nahr. Untersuch.*, 1896, p. 173.

† *Journ. Pharm. Chim.*, 1898, p. 540.

‡ *Jahresber. Nahr. Genussm.*, 1891, p. 38.

If dextrins and dextrose are present as well as glycogen, Niebel advocates the use of Landwehr's method. The broth, prepared in the manner described above, is neutralised and freed from albuminous substances by the addition of a little zinc acetate. The filtrate and washings are heated on the water-bath with a sufficient quantity of a concentrated solution of ferric chloride, after which a concentrated solution of sodium hydroxide is added, drop by drop, until all the iron is precipitated. The precipitate is rapidly filtered off, washed with hot water, and dissolved in concentrated acetic acid. The cold solution, to which hydrochloric acid has been added, until a yellow coloration is obtained, is poured into alcohol, and the flocculent precipitate of glycogen is collected, washed and dried as described above.

When the glycogen has undergone decomposition, as in the case of sausages which have been kept for some time, Niebel converts the whole of the carbohydrates present into dextrose, and determines the total amount of reducing substances in the flesh by means of Fehling's solution. In sausages containing no horseflesh he found not more than 0·7 per cent. of dextrose, while when horseflesh was present the amounts found varied from 1·189 to 3·707 per cent., and in these glycogen could, as a rule, be identified. In the absence of added starch or sugar, Niebel regards the presence of horseflesh as proved when the total amount of carbohydrates (expressed as dextrose) exceeds 1 per cent., calculated on the fat-free dry substance, and when the flesh itself is of a brownish-red colour. Obviously, this method is useless in the case of sausages containing bread or other amylaceous substances.

Bujard[*] regards Mayrhofer's method (p. 131) as more suitable than that of Niebel for the determination of glycogen. The flesh is dissolved in aqueous potassium hydroxide, proteid substances precipitated by means of hydrochloric acid and Nessler's reagent, and the glycogen, after precipitation by the addition of alcohol to the clear filtrate, is washed on a weighed filter with dilute alcohol and ether, and dried at 110° C.

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[*] *Forschungs Ber.*, 1897, iv. p. 47.

The results given in Table I. were obtained recently by this method, whilst those in II. were obtained some time ago by Niebel's method :—

TABLE I.

	Water. Per Cent.	Per Cent. Glycogen Direct.	
		Niebel.	Mayrhofer.
Horseflesh, ...	74·44	0·440	0·445
"	74·87	0·600	0·520
"	76·17	1·827	1·727
"	76·00	0·592	0·610
Red sausage (Knackwurst),	69·26	...	0·038*
Pork sausage, . . .	67·25	...	0·240*
Veal, ...	74·6	...	0·086
Pork, ...	75·0	...	0·186

TABLE II.

	Per Cent.		
	Water.	Glycogen.	Glycogen on Dried Substance.
Horseflesh, ...	61·83	0·846	2·24
"	72·90	0·174	0·64
"	70·47	1·366	4·62
"	71·84	0·59	2·09
" (smoked), .	43·00	0·108	0·19
Beef (ox), ...	73·62	0·206	0·74

* In these pepper-starch could be detected microscopically, and on testing with iodine only the blue starch reaction could be obtained, whilst in all the other cases the glycogen reaction was marked.

TABLE II.—*continued.*

	Per Cent.		
	Water.	Glycogen Direct.	Glycogen on Dried Substance.
Beef, ...	75·55	0·018	0·073
Veal, ...	76·12	0·346	1·44
,, ...	74·47	0·066	0·25
Pork, ...	54·05	trace	trace
,, ...	66·29	...	...
Horse Sausages.			
Red sausage, . . .	70·04	0·504	1·68
Liver ,, . . .	67·00	1·762	5·34
Salami, ...	33·60	0·034	0·05
Sausages.			
Salami, ...	20·00	trace	trace
Thuringian, . . .	12·93	,,	,,
,, . . .	29·16	,,	,,

From these results Bujard concludes that only in exceptional cases (where the amount is large) can the glycogen be taken as conclusive of the presence of horseflesh, especially when the latter is mixed with other kinds of flesh.

If we take into consideration the results of these different chemists, the reaction of glycogen with iodine and the quantitative determination of glycogen must be regarded as giving uncertain conclusions as to the presence of horseflesh. Apart from the fact that glycogen appears normally in certain organs of other animals, it has been shown that its formation and distribution throughout the body is influenced by the food given to the animal, and also that the quantity is subject to considerable variation in certain diseased conditions. On the other hand, in old sausages the glycogen may undergo decomposition, and a negative result be obtained with the tests, when horseflesh is actually present. Hence the results obtained by these and similar methods must only be taken as corroborative evidence.

v. *The Form of the Fat Cells.*—According to Jungers[*] the fat cells of the different animals used for food show distinct differences in external form, which are especially marked in the case of the horse. This difference can also be observed in the fat cells of apparently fat-free flesh, and can be used for the detection of horseflesh in mixtures. In boiled and smoked sausages, however, it is only possible to find unaltered fat cells by taking the test from the centre of the sample. The nature of this characteristic difference is not described.

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[*] *Jahresber. Nahr. u. Genussm.*, 1894, p. 64.

vi. *Examination of the Fat.*—The fat extracted by one of the methods given on p. 83 is examined by the usual methods, and the results compared with the figures of the constants in the tables on pp. 52–59.

Crystallisation from Ether.—When the sausage is composed entirely of pork the fat on crystallisation from ether will, as a rule, give the characteristic chisel-shaped crystals. Horse fat, on the other hand, is very soluble in ether, but by using very small quantities of solvent, crystals resembling those of beef stearin (pp. 50 and 58) can be obtained.

Iodine Value.—Since the mean iodine value of beef fat is about 55, whilst that of horse fat is about 83, a determination of this constant is valuable when the sausage is composed of only one of these kinds of flesh, which, however, is not often the case.

R. Frühling,* in his experiments on this point, determined the iodine value of the fat from different sausages. The finely divided substance was boiled for a considerable time with water, and after cooling, the layer of the fat on the surface of the liquid was removed, filtered, and its iodine value determined by Hübl's method.

The results obtained with the fat thus extracted were :—

	Iodine Value.
Sausage made from pure horseflesh, ...	72·5
" consisting of horseflesh with 15 per cent. of pork, .	62·3
" " " 50 " .	57·2

Since lard has an iodine absorption of from 56·9 to 63·8 (Benedikt), it is obvious that this method would lead to no certain conclusion in the case of mixtures.

Examination of the Intermuscular Fat.—The fat within the muscular fibre was first examined by Nussberger.† The fat was extracted from the muscle of various kinds of horseflesh (kidneys, ham, etc.) by means of ether, and iodine values of from 80 to 94 obtained, the mean being

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* *Zeit. angew. Chem.*, 1896, p. 352.

† *Chem. Rundschau*, i. p. 61.

84. The iodine values obtained were probably too low on account of the presence of other substances, besides fat, extracted by the ether.

Bremer* carries Nussberger's method a step further, and determines the iodine value of the more fluid portion of the fatty acids obtained from the intermuscular fat. The sausage mass, from which all visible fat has been removed, is finely minced, mixed with water, and heated for about an hour on the water-bath. The fat rising to the surface is poured away with the water, and the flesh, after having been washed several times with hot water, is dried at 110° C. for twelve hours, and extracted for several hours with a petroleum spirit of low boiling-point. Part of the intermuscular fat thus obtained is used for the determination of the iodine value, refractive index, and Reichert-Meissl value. The remainder is saponified, the excess of alkali neutralised with acetic acid, and the alcohol evaporated on the water-bath. The soap is dissolved in hot water, the liquid fatty acids separated as zinc salts by Jean's method (p. 98), and the iodine value of the soluble zinc salts, or of the acids liberated from them, determined as described on page 99.

The following table gives the results which Bremer obtained:—

	Iodine value of inter-muscular fat.	Iodine value of liquid acids of the fat.
1. Horseflesh sausage without bacon, . .	75·8	108·1
2. ,, ,, with about 6 per cent. of bacon, ...	74·0	104·1
3. Horseflesh sausage with about 22 per cent. of bacon well smoked, ...	53·7	92·4
4. Horseflesh cervelat sausage with about 69 per cent. of bacon, ...	74·1	102·1
5. Ordinary sausage with some bacon, . .	57·6	94·2
6. Thuringian cervelat sausage with about 65 per cent. of lard, ...	64·3	95·8
7. Mixture of 1 and 5 in equal parts, . .	66·4	103·1
8. Mixture of 4 and 6 in equal parts, . .	65·2	99·5

Bremer also found that when horseflesh was present the petroleum spirit extract had a red to reddish-brown colour, and that even the liquid fatty acids had a more or less pronounced reddish-yellow shade. On the other hand, bull's flesh gave a similar colour, so that this fact can only be used as a confirmatory test. When, however, this coloration is obtained, when at the same time glycogen is detected, and when the iodine number of the intermuscular fat exceeds 65, and that of the liquid fatty acids is considerably over 95, there can, in Bremer's opinion, be but little doubt as to the presence of horseflesh.

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* *Forschungs Ber.*, 1897, iv. p. 5.

The Artificial Coloration of Sausages.—Sausages are artificially coloured either with the object of concealing an addition of starch or bread, or of improving the colour of the meat, and in some cases disguising its condition. When fresh beef is exposed to the atmosphere it soon changes its colour, and the bright red, due to the oxyhæmoglobin, becomes dark brown and eventually yellowish-brown or grey. Similar alterations take place in lighter coloured flesh, such as veal and pork, though they are not so pronounced as in beef. When the exposed meat is in the finely-minced state in which it is used for sausages, these changes occur with great rapidity. When decomposition, whether of an acid or alkaline nature (*cf.* pp. 74–76), has commenced, the surface of the meat often assumes a bluish or greenish tint.

On being heated to 70° or 80° C. the hæmoglobin of the flesh is decomposed, and hæmatin, which has a brown colour, is formed. Hence red flesh (beef, mutton) becomes dark brown on cooking, while the lighter coloured meats (*e.g.*, veal, pork, and fowl), which contain comparatively little hæmoglobin, do not show this change in colour, but become grey.

In order to regain the original colour many sausage manufacturers are in the habit of adding a trace of some colouring matter such as cochineal, carmine, or an aniline dye, and this has been a common practice in Germany for the last fifty years. Lately, however, the question has received considerable attention, and there is a growing opinion that since the practice offers great facility for disguising unsound flesh, it ought to be altogether forbidden. As an instance in point it may be mentioned that H. Bremer * recently met with a cervelat sausage coloured with carmine, which when cut had all the appearance of sound flesh, but on further examination was found to be quite unfit for food, the acid value of the fat being 76·0.

The Microscopical Detection of Colour.—G. Marpmann † recommends the following method of examination:—A section of the sausage, about 1 cm. thick, is thoroughly moistened with 50 per cent. alcohol, and examined under the microscope. When only traces of colouring matter are present, the substance is dehydrated in xylol, which is expelled by means of carbon tetrachloride, and the mass placed in cedar oil. As thus prepared it is transparent, and colouring matters present can readily be recognised. Fuchsin, magenta red, diamond red, carmine, logwood, and orchil stain the cell substance, while acid aniline colours dye the liquid in the cell. In some instances (*e.g.*, with safranin) the colouring matter must be concentrated, and wool or animal tissue placed in the concentrated solution. The finely divided sausage is digested with 50 per cent. alcohol, the liquid (freed from fat) evaporated to a few drops, and some undyed sausage placed in this solution. The muscular fibres and the fat cells are then stained deeply. Marpmann states that safranin is largely employed for dyeing cervelat sausages.

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* *Forschungs Ber.*, 1897, 4, p. 45.

† *Zeit. angew. Mikrosk.*, 1895, p. 12.

The sausage should also be extracted with ammoniacal water, which is a better solvent than alcohol for many of the colours used as flesh-dyes. Marpmann regards with suspicion all sausages which remain coloured after being kept for two hours in 50 per cent. alcohol, since normal flesh is decolorised under these conditions.

Action of certain Dyes on Flesh Proteids.—The following table of Marpmann shows the behaviour of different flesh proteids on treatment with certain aniline colours:—

	Corallin.	Eosin.	Phloxin.	Congo Red.	Safranin.
Albumin, .	Bluish rose.	Raspberry red.	Raspberry red.	...	...
Myosin, .	...	,,	,,	...	...
Peptone, .	Orange yellow, afterwards decolorised.	...	...	Orange precipitate.	Decolorised
Nucleo-albumin, .	...	Orange.	...	...	...
Syntonin, .	Reddish.	...	...	Brown.	Yellow.
Alkaline Albuminate,	...	Violet-rose.	Reddish.	Rose.	Yellowish.
Fibrin, .	Red.	Rose.	Rose.	Red.	Yellowish red.

In addition to alcohol, various solvents have been recommended for the extraction of artificial colouring matter in sausages, such as amyl alcohol, or a mixture of glycerin and alcohol. According to Bremer,* cases are frequently met with in which the artificial colour can be detected microscopically, but cannot be extracted with any of these solvents. In such cases he advocates the use of equal parts of glycerin and water, as recommended by Klinger and Bujard. The finely divided substance is heated for several hours on the water-bath, with two volumes of this mixture

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* *Forschungs Ber.*, 1897, p. 216.

(slightly acidified), the yellow solution freed from fat and filtered, and the colouring matter precipitated as a lake by the addition of alum and ammonia.

On placing the test-tube before the spectroscope, the absorption lines of carmine-lake, lying between *b* and *D*, may then be identified. Since the acid solution of the sausage colouring matter is yellow, while carmine-lake gives a red solution with hydrochloric, nitric, and tartaric acids, Bremer suggests that the carmine in such sausages must be present in some other form than lake, possibly combining with the preservative to form a compound insoluble in alcohol.

The Action of Nitre on Natural Colouring Matters in Flesh.—In the examination of a number of American sausages, Weller and Riegel * met with three which were of a suspicious colour, and from which the colouring matter could be extracted with glycerin and water, with amyl alcohol, with alcohol and with ether, all the solvents being coloured from light red to dark red. On extracting the meat with acidified glycerin and water, as directed by Bremer, a bright red solution was obtained, but this, when evaporated on the water-bath, after the addition of ammonia, remained unaltered. The colouring matter also dissolved readily in acidified alcohol and amyl alcohol, but on evaporating the solution in contact with wool-fibre, it was not possible to fix the colour on the wool, even in the presence of aluminium salts.

Experiments were then made to determine whether any of the salts with which the sausages were strongly impregnated had any effect on the blood-colouring matter, and from the results the conclusion was arrived at that the colouring matter extracted from these sausages was due to this cause. It was found that flesh containing blood, when dried with sodium chloride, potassium chloride, sodium nitrate, or mixtures of these salts, yielded only minute traces of colour to the solvents. But, on the other hand, deep red solutions were obtained from pig's flesh containing blood, which had been dried with potassium nitrate; and these behaved in the same way as the coloured solutions obtained from the sausages. The spectroscopical examination showed that the oxyhæmoglobin had undergone alteration, the acidified glycerin solution, diluted with water, giving a spectrum similar to that of methæmoglobin (see p. 39).

It was further proved that the alteration of the oxyhæmoglobin was not due to the flesh fibrin being brought into solution by the nitre, and it appeared to be a special characteristic of swine's blood hæmoglobin. In one experiment in which calf's blood was dried with nitre, only slight traces of colour could be obtained after two days' extraction with ether. The fibrin from normal venous blood was found to be readily soluble in a solution of nitre, whereas that from arterial or diseased blood (especially in the case of the ox) was frequently insoluble.

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* *Forschungs Ber.*, 1897, p. 204.

From this investigation Weller and Riegel concluded that Bremer's process (p. 143) is only reliable when the colouring matter can be precipitated from its solution as a lake, and identified chemically or spectroscopically. But since many vegetable colours, which are soluble in water but insoluble in alcohol or amyl alcohol, cannot be precipitated as lakes, Bremer's method, like the others, may often fail. A microscopical examination, too, may be inconclusive when the sausage has been coloured with an aqueous solution of a vegetable colouring matter thoroughly distributed, though in such cases the reactions given by the aqueous extract with ferric chloride, lead acetate, calcined magnesia, manganese peroxide, sodium bicarbonate, etc., may give useful indications. Weller and Riegel regard the official method of the Berlin Police Council (extraction with glycerin and water for fifteen minutes in the water-bath) as useless in the light of their experiments.

With reference to these conclusions, E. Spaeth[*] states that he is unable to confirm their observation on the action of nitre on the colouring matter of the blood. He has made numerous experiments with uncoloured sausages, and only in one, which had been heated with a large quantity of that salt, did the extract show a faint yellowish-red colour.

Nitre is often added to pickling beef with the object of preserving the natural colour of the flesh. But Serafini[†] found that even when added in as large an amount as 5 per cent. it had neither antiseptic nor colour-preserving properties. He considered that, taking into account the injurious effects of its continued use, it ought to be forbidden altogether in sausages.

Extraction of the Colour with Sodium Salicylate.—E. Spaeth[*] finds that the ordinary artificial colours used in sausages can be most readily extracted by warming the finely divided substance for a short time on the boiling water-bath with a 5 per cent. solution of sodium salicylate. When a mixture of glycerin and water is used as the solvent it is often necessary to extract hard sausages for hours, and when only a trace of colour has been added it may not even then dissolve.

According to Spaeth, aniline colours and carmine are almost exclusively used for colouring sausages, while vegetable colouring matters are but rarely employed. On the addition of ammonia to the aqueous extract, red precipitates may be obtained, consisting of calcium and magnesium phosphate and possibly aluminum hydroxide, carrying down traces of an aniline colour mechanically. Hence, a further examination of the precipitate is required before the presence of carmine is regarded as proved.

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[*] *Pharm. Centralb.*, 1897, 38, p. 884.

[†] *Jahresber. Nahr. Genussm.*, 1892, p. 45.

For a method of identifying natural and artificial colouring matters alone and in admixture with others, see a paper by Rota in the *Analyst*, 1899, p. 41.

CHAPTER VIII.

THE PROTEIDS OF FLESH.

Definition.—It is not an easy matter to find a simple and comprehensive definition for the proteids—the most important of the constituents of flesh. Some of those proposed are cumbersome, and involve the use of long periphrases, while others are inexact. One of the most concise is the recent one of Wroblewski, who describes them as bodies which, on complete decomposition with acids, yield as final products ammonia, nitrogenous organic bases (such as lysine, arginine, etc.), and amido-acids (such as leucine, tyrosine, etc.). This definition, however, is a somewhat arbitrary one, and as it probably excludes the peptones from the class of proteids, cannot be regarded as altogether satisfactory.

Mulder's 'Proteine' Theory.—Mulder found that by the action of potassium hydroxide on the substances we now name 'proteids,' products (albuminates), which he regarded as identical in each case, were obtained. Taking this into account with the fact that the proteids themselves contained the same elements in nearly the same proportion after deducting the ash, he conceived the theory that these complex nitrogenous constituents of blood, flesh, and other organic tissues and fluids were all compounds of a definite substance with phosphates and other salts. To this substance he gave the name of 'proteine' (πρωτειον = pre-eminent), since he regarded it as the primary constituent of all animal tissues.

Thus albumin was protein + phosphates + sulphur.
„ fibrin „ + „ + 2 „
„ hair, horn „ + ammonia + 3 water,
and so on.

Liebig and other chemists showed the incorrectness of this theory, and only the name 'proteid' has survived.

Classification of Proteids.—There is too little variation in the elementary composition of different proteids for any scheme of classification to be based upon it, and advantage has therefore been taken of the difference in solubility of different individuals, and the nature of the products yielded by them on decomposition. Two recent schemes which have much in common are those of Chittenden* and Wroblewski.† In both there are three main groups:—I. Albuminous bodies (Wroblewski), Simple Proteids (Chittenden); II. Compound Albuminous Substances (Wroblewski), Compound Proteids (Chittenden); III. Albuminoid Substances.

In Wroblewski's scheme the albumoses and peptones are placed with the albuminoid substances in the third group, whilst in Chittenden's scheme they are placed in the first group, and enzymes are not included in the classification.

The scheme on pp. 150, 151 is based on both these systems of classification, the general arrangement being that of Wroblewski, and the classification according to solubility, especially in the case of the albumoses and peptones, being due to Chittenden.

Albuminous Substances.

The proteids classified in this group are related more or less closely to fresh or coagulated white of egg. They contain carbon, hydrogen, nitrogen, oxygen, and sulphur in only slightly varying proportion. According to Neumeister, the percentage variation is within the following limits:—

	Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Sulphur.
Maximum, .	55	7·3	17·6	24·0	2·4
Minimum, .	50	6·5	15·0	19·0	0·3
Mean, . .	52	7·0	16·0	23·0	2·0

The molecular weights of albuminous substances appear to be very high, from the results of the analysis of their metallic compounds and cryoscopic determinations by Raoult's method. Thus Sabanejeff assigns to purified egg albumin a molecular weight of 15,000, and Schützenberger gives the formula $C_{240}H_{892}N_{65}O_{75}S_{8}$ to the same substance.

The composition of several of the important members of this group is shown on the next page, upon which is given an interesting table, showing the percentage composition of some of the more important naturally-occurring proteids, selected from the longer one of Chittenden *:—

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* *Medical Record*, 1894, p. 45.

† *Berichte d. d. Chem. Gesells.*, 1898, p. 3045.

COMPOSITION OF NATURALLY-OCCURRING PROTEIDS.

Proteid.	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.	Phosphorus.	Ash.	Origin.	Authority.
Serum albumin, .	53·05	6·85	16·04	1·77	22·29	...	0·57 1·84	Blood of horse.	Hammarsten.
Egg albumin, . .	52·33	6·98	15·89	1·83	22·97	...	1·11	Non-coagulated.	Chittenden and Bolton.
Lacto-albumin, .	52·19	7·18	15·77	1·73	23·13	...	...	Cow's milk.	Sebastien.
Myosin, . .	52·82	7·11	16·77	1·27	21·90	...	1·45	Muscle.	Chittenden and Cummins.
Paraglobulin, . .	52·71	7·01	15·85	1·11	23·24	...	0·30	Horse blood.	Hammarsten.
Fibrinogen, . .	52·93	6·90	16·66	1·25	22·26	...	1·75	Do.	Do.
Coagulated proteid, .	52·33	6·98	15·84	1·81	23·04	...	0·27	Egg albumin.	Chittenden and Bolton.
Fibrin, . . .	52·68	6·83	16·91	1·10	22·48	...	0·56	Blood of horse.	Hammarsten.
Oxyhæmoglobin, .	53·85	7·32	16·17	0·39	21·84	...	0·43	Dog's blood.	Hoppe Seyler.
Oxyhæmoglobin, .	54·71	7·38	17·43	0·48	19·60	...	0·39	Pig's blood.	Hüfner.
Mucin, . . .	50·30	6·84	13·62	1·71	27·53	...	0·33	Snail.	Hammarsten.
Nucleïn, . .	50·60	7·60	13·18	...	...	1·89	...	Human brain.	Jaksch.
Casein, . . .	52·96	7·05	15·65	0·71	22·78	0·84	...	Cow's milk.	Hammarsten.
Gelatin, . .	49·38	6·81	17·97	0·71	25·13	...	1·26	Connective tissue.	Chittenden and Solley.
Elastin, . .	54·24	7·27	16·70	0·30	21·79	...	0·90	Neck-band.	Chittenden and Hart.
Keratin, . .	49·45	6·52	16·81	4·02	23·20	...	1·01	Rabbit's hair.	Kühne and Chittenden.
Proteose, . .	52·13	6·83	16·55	1·09	23·40	...	0·79	Hemialbumose, urine.	Kühne and Chittenden.

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* *Medical Record*, 1894, p. 450.

CLASSIFICATION

GROUP I. Albuminous Substances.	GROUP II. Compound Albuminous Substances.
1. *Soluble in water. Coagulable by heat or long contact with alcohol.* Albumins: Egg albumin. Serum albumin. Muscle albumin. Plant albumins, etc.	1. *Compounds of a proteid with an iron-containing pigment. Soluble in water, and coagulated by heat and alcohol.* Hæmoglobin. Oxyhæmoglobin. Methæmoglobin.
2. *Insoluble in water, but soluble in salt solutions. More or less coagulated by heat.* Globulins: *a.* Soluble in dilute and saturated solutions of sodium chloride. Vitellins. *b.* Soluble in dilute solutions of sodium chloride, but precipitated on saturation with that salt. Egg globulins. Serum globulins. Lacto-globulin. Cell globulins. Fibrinogen. Myosin, etc.	2. *Compounds of a proteid with a member of the carbohydrate group. Insoluble in water. Soluble in very weak alkalies.* Mucins. Mucoids.
3. *Insoluble in water and salt solutions. Soluble in dilute alcohol.* Albuminous substances chiefly of vegetable origin: Zein, Gliadins.	3. *Compounds of a proteid with nuclëic acid. Phosphorised bodies yielding on decomposition metaphosphoric acid.* Insoluble in water and acid pepsin solution, but more or less soluble in alkalies. Nuclēins.
4. *Insoluble in water, salt solutions and alcohol. Soluble in dilute acids and alkalies.* *a.* Coagulated by heat, when suspended in a neutral fluid. Acid albumins: Syntonin, and the like. Alkali albumins: Albuminates. *b.* Not coagulated by heat in a neutral fluid. Glutenins.	4. *Compounds of proteids with nuclēins. Very soluble in dilute alkalies.* Nucleo-albumins of cell-protoplasm. Cell nuclei, etc. Caseins.
5. *Insoluble, or nearly so, in water, salt solutions, and alcohol. Soluble in strong acids and alkalies, and in acid pepsin and alkaline trypsin solutions.* Coagulated albuminous substances: Fibrin. Coagulated white of egg, etc.	5. Amyloids.
	6. Histones?

OF PROTEIDS.

Group III. Albuminoid Substances.		
Class I. Structural Substances.	Class II. Derivatives of Albuminous Substances. Proteoses, Peptones, etc.	Class III. Enzymes.
1. *Soluble in boiling water, and yielding on decomposition leucine and glycocoll.* Collagenes: Gelatin. Glue, and the like.	1. *Soluble in water. Not coagulated by heat or alcohol.* *a.* Proto- and Deutero-proteoses: Protoalbumose. Deuteroalbumose. Globuloses. Elastoses. Myosinoses. *b.* Peptones: Amphopeptones. Hemipeptones. Antipeptones.	1. *Proteolytic:* Pepsin. Trypsin. Papayotin, and the like.
		2. *Amylolytic:* Diastase. Invertin, and the like.
2. *Insoluble in boiling water. Yielding on decomposition much tyrosine, with leucine and glycocoll. Slowly hydrated by boiling dilute acids and by pepsin with HCl.* Elastins.	2. *Insoluble in water. Soluble in dilute salt solutions. Precipitated by saturation with NaCl.* Hetero-proteoses: Hetero-albumoses. Hetero-globuloses. Hetero-myosinoses, etc.	3. *Fat-Decomposing Enzymes:* Steapsin, and the like.
		4. *Glucoside-Decomposing Enzymes.*
3. *Insoluble in water, dilute acids and alkalies, and in acid pepsin and alkaline trypsin solutions. On decomposition yield leucine and tyrosine.* Keratins. Neurokeratins.	3. *Insoluble in water, salt solutions, and alcohol. Soluble in dilute acids and alkalies.* Dysproteoses. Antialbumids.	5. *Amide-Decomposing Enzymes:* Urase, and the like.
		6. *Coagulating Enzymes:* Rennet, and the like.

Albumins, of which egg albumin may be taken as the type, are soluble in water, and coagulate on heating. Egg albumin coagulates at about 72° C., and has a specific rotation of $-35{\cdot}5$. When white of egg is dried at 100° C. it loses about 88 per cent. of its weight. In preparing ordinary commercial albumin, white of egg is evaporated at a low temperature, leaving light yellow flakes. Or sometimes the fibrin, which is also present in small quantity, is previously removed by beating and filtering through a cloth.

Globulins are closely allied to albumins, but differ from them in their behaviour towards salt solutions. They dissolve in dilute solutions of sodium chloride, but are as a rule precipitated by saturating the liquid with sodium chloride or magnesium sulphate.

The **Vitellins**, of which representatives are found in egg-yolk and in the eyes of fish, differ from the globulins proper in not being precipitated by saturation with sodium chloride.

Acid Albumins.—These are compounds of hydrochloric or acetic acid with an albumin or globulin. They are produced as the first stage in the hydrolysis of these substances by means of pepsin. Myosin, for example, in the digestive process first forms an acid albumin or *syntonin*. Like globulins, they are precipitated by saturating their solution with sodium chloride or magnesium sulphate.

Alkali Albumins or **Albuminates** are produced by the action of alkalies on albumins or globulins. They are soluble in alkalies, but not in neutral liquids.

Coagulated Albumins.—Under the influence of heat, or long contact with alcohol, or in some cases by the action of enzymes, albuminous substances become converted into a peculiar modification which is exceedingly insoluble. Types of these are coagulated white of egg and fibrin from fibrinogen. The temperature of heat coagulation varies with the nature of the salts in the liquid and with the concentration of the solution (*cf.* p. 162). It is curious that coagulation cannot be brought about by boiling a solution of an albuminous substance to which a trace of formaldehyde has been previously added.

Compound Albuminous Substances.

These consist of proteids, whose molecule is composed of a simple albuminous substance in combination with another substance often of a non-proteid nature.

Hæmoglobins.—In the *hæmoglobins* there is a colouring matter group which contains iron (see p. 37).

Mucins.—*Mucins* and *Mucoids* are representatives of compounds of albuminous substances with a carbohydrate.

Mucins are found in secretions of various glands and on the skin of the snail. They can be precipitated from their solutions in the absence of salts by means of acetic acid or a mineral acid. The precipitate is insoluble in an excess of acetic acid, a property which is made use of in the separation of mucins from albumins. According to Neumeister they have the following composition:—nitrogen, 11·7 to 12·3; carbon, 48·3 to 48·8; oxygen, 31·3 to 33·6; and sulphur, about 0·8 per cent.

By long-continued boiling with dilute mineral acids, or by the action of superheated steam, mucins are converted into syntonins and eventually peptones, while substances of a carbohydrate nature are liberated (*cf.* p. 24).

Mucoids are closely allied to mucins. They have been isolated in small quantity from the white of birds' eggs, and from the cornea of the eye.

Hyalogens are substances which are often grouped with the mucoids. By the action of dilute potassium hydroxide, they are converted into very insoluble substances known as *hyalins*. Hyalogens are found in the skin of the serpent and in the bladder of the echinococcus (*cf.* p. 243).

Nucleïns.—The composition of a representative nucleïn is given in the list on p. 149. See also p. 6.

Albuminoid Substances.

I.—Structural Substances.

Collagene is a widely distributed substance, forming, as it does, a principal part of the connective tissue and the organic substance of bone. In Neumeister's opinion it is probably produced in the animal system by the decomposition and oxidation of albuminous substances.

Its mean composition is:—Carbon, 50·75; hydrogen, 6·47; nitrogen, 17·86; oxygen, 24·32; and sulphur, 0·6 per cent.

Collagene, unlike the albuminous substances, does not yield tyrosine on hydrolysis, the end products of the decomposition brought about by boiling hydrochloric acid being leucine, aspartic acid, glutamic acid, and glycocoll. The sulphur, which it contains, appears to be in a much closer state of combination than is that of albumin.

Gelatin.—When collagene or substances containing it are boiled with water, the collagene is hydrated and dissolves in the form of gelatin or glue, the latter being an impure gelatin. The elementary composition of gelatin is shown on p. 149, and the composition of the first products of its hydrolytic decomposition on p. 181.

Gelatin is not precipitated by mineral acids, by potassium ferrocyanide with acetic acid, or by salts of lead or copper. It is, however, precipitated by most of the other reagents for proteids. Bromine or chlorine precipitate it quantitatively, and it combines with tannin in the presence of salt to form a characteristic insoluble compound (leather). Mercuric chloride precipitates it in the presence of hydrochloric acid.

Gelatin undergoes hydrolysis with great readiness. On boiling it for a short time with very dilute acid, or for a long time with pure water, it loses its gelatinising power through its conversion into gelatose.

Elastin.—This proteid is the main constituent of the elastic tissue. In the analysis of its elementary composition (p. 149) sulphur is given as one of its constituents, but later analyses of pure elastin have shown that it does not contain sulphur.

It can be brought into solution by treatment with superheated steam, or by boiling it for several hours with dilute mineral acids or strong alkali (*cf.* p. 24).

Keratins are found in such parts of the animal system as the hair, horns, nails, and feathers. They contain a large proportion of sulphur (from 3 to 5 per cent.), while the oxygen is less than that of true albuminous bodies. They are remarkably insoluble, but can be brought into partial solution by the action of superheated steam or by boiling with alkali.

II.—DERIVATIVES OF ALBUMINOUS AND STRUCTURAL ALBUMINOID SUBSTANCES.

Proteoses.—This word is used as a convenient generic term for certain products of the hydrolysis of native proteids in which the decomposition, whether brought about by acids, superheated steam, or proteolytic enzymes, has only proceeded to a certain extent. Thus it includes the albumoses, derivatives of albumin; fibrinoses from fibrin; caseoses from casein, etc. Not unfrequently, however, all such products are termed 'albumoses,' since the latter have received the most study, and may be regarded as typical proteoses.

Albumoses.—For the want of more accurate knowledge we group together under this name a large number of albuminous derivatives with a few characteristics in common. Further subdivision can be effected into groups which behave differently with different solvents; but proteolysis is not a simple process, and at any given stage of the decomposition each group contains substances with ever-varying composition, until finally, with the continuation of the hydrolysis, the products are gradually broken up into substances of a simpler composition, lower molecular weight, and greater solubility, which can be grouped together as peptones.

This is doubtless the reason why in many analyses of meat extracts, peptones have been found by one chemist and not by another. For instance, in methods of analysis in which alcohol is used as the precipitating agent, those products which are most soluble, or, in other words, require the greatest addition of alcohol for their precipitation, are returned as peptones; whereas, if saturation with zinc sulphate were used, they might be partially precipitated together with the derivatives more closely related to the original proteid, and be classed with the albumoses.

Old Names for Albumoses.—Some confusion has also been caused by the fact that formerly all the products of peptic digestion were called peptones. When a differentiation between the higher and lower products had been effected, Kühne gave to the former the name of *propeptones*, while Meissner termed them *a-peptones*. Eventually the name *albumoses* was adopted by Kühne.

General Properties.—Albumoses differ from native albuminous substances in being much more soluble, and in not being coagulated by heat or by alcohol, though they can be precipitated by the latter. They contain less carbon, but more oxygen, and have much lower molecular weights. They are slightly diffusible, while albumin proper is completely indiffusible.

Like the native proteids they are precipitated from their aqueous solutions by saturation with zinc sulphate or ammonium sulphate. They can also be precipitated by chlorine or bromine, nitric acid, mercuric chloride, phosphotungstic acid, tannic acid, picric acid (primary albumoses), trichloracetic acid, and less readily by a solution of mercuric iodide and potassium iodide in the presence of hydrochloric acid.

Subdivision of Albumoses.—The different albumoses formed in the earlier stages of the decomposition may be grouped under *protoalbumoses* and *heteroalbumoses*, which collectively form the *primary albumoses*. From each group of primary albumoses further hydrolysis, as in the process of peptic digestion, forms *deuteroalbumoses*, and finally peptones.

This is shown in the scheme of Neumeister, representing the action of pepsin and hydrochloric acid,* without reference to the hemi- and anti-groups of the molecule given on the next page.

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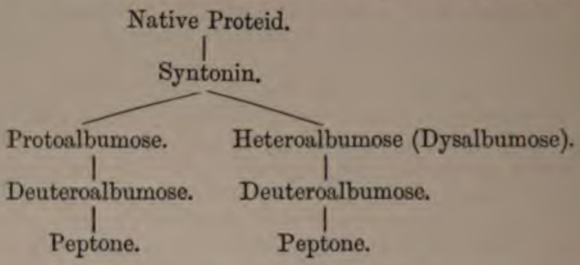
* *Lehrbuch Physiol. Chem.*, p. 231; *cf.* p. 180.

DIAGRAM OF THE ACTION OF PEPSIN ON PROTEIDS.



Primary Albumoses.—These are precipitated, though not completely, by neutralising the solution, and saturating it with sodium chloride, which gives a white precipitate, dissolving on heating, and reappearing on cooling. They are also precipitated by nitric acid, while deuteroalbumoses give no precipitate until the liquid has been first saturated with common salt.

Other precipitants for primary albumoses are potassium ferrocyanide with acetic acid, and copper sulphate, though these also sometimes precipitate small quantities of deuteroalbumose.

Protoalbumoses are soluble in distilled water, and in dilute solutions of salt, and are partially precipitated by saturating an acidified solution with salt. They are also precipitated by mercuric chloride and by copper sulphate.

Heteroalbumoses are insoluble in distilled water, but dissolve in weak solutions of salt. They are precipitated like the globulins by pouring their neutralised solution into a large volume of pure water, or by saturating the solution with sodium chloride. They are also precipitated by copper sulphate and by mercuric chloride (in acid solutions).

Dysalbumose.—By being left for a long time in contact with water, or by drying, heteroalbumoses are converted into a peculiar insoluble modification known as *dysalbumose*, which can be partially reconverted into heteroalbumoses by treatment with dilute acid or sodium hydroxide.

Deuteroalbumoses are much more closely allied to the peptones than are primary albumoses. They are soluble in water and solutions of salts, and are not precipitated by saturating their solution with sodium chloride. Nitric acid precipitates them only in the presence of an excess of salt, and the precipitates do not dissolve so readily on heating as those of the primary albumoses.

Chittenden [*] gives the following method of isolating them from the primary compounds in the absence of peptones. The solution is neutralised and saturated with sodium chloride, which partially precipitates the primary albumoses. On adding acetic acid, drop by drop, to the filtrate, the residual protoalbumoses are precipitated together with a small amount of deuteroalbumose. From the filtrate from this precipitate the deuteroalbumoses can be obtained in a pure condition by dialysing out the salt and acid, concentrating the liquid, and precipitating the proteid with alcohol.

It is not an easy matter to completely precipitate the whole of the deuteroalbumoses in a solution of mixed albumoses, and Kühne states that long-continued boiling in the alternately neutral and alkaline saturated liquid is necessary.

S. Fränkel [†] proposes to separate deuteroalbumoses by means of cupric sulphate, and thus to avoid the difficulty of removing large quantities of salts by dialysis. According to Neumeister, this reagent gives a voluminous precipitate, with a solution of 1 : 500, and a turbidity with a solution of 1 : 1000 of deuteroalbumose containing protoalbumose, but gives no sign of turbidity with pure deuteroalbumose. On adding a dilute solution of cupric sulphate to the albumose solution, a tough coherent precipitate is formed, while any turbidity left in the solution generally disappears after a few hours. The copper is removed from the solution by adding a hot saturated solution of barium ferrocyanide, until a few drops of the liquid on filtration show only a trace of copper. At this stage the liquid is acidified with acetic acid, warmed, filtered, and the filter washed. Barium ferrocyanide solution is added, drop by drop, to the filtrate so long as a red precipitate is formed, then barium acetate to remove the sulphuric acid. Finally the solution is concentrated and poured into strong alcohol, and the deuteroalbumose dehydrated with absolute alcohol, and washed with ether.

Schrötter's Albumose.—H. Schrötter [‡] isolated from Witte's peptone an albumose, or group of albumoses, in the following manner:—The soluble impurities were extracted with methyl alcohol, and the residue dissolved in water acidulated with sulphuric acid, and treated with zinc dust and sulphuric acid. After several days the liquid was warmed on the water-bath, and, after the removal of the sulphuric acid, filtered, concentrated, and evaporated *in vacuo* over sulphuric acid. The residue was exhausted with hot methyl alcohol, the extract concentrated, and the albumose precipitated with absolute ether.

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[*] *Medical Record*, 1894, p. 485.

[†] *Monatshefl. f. Chem.*, 1897, p. 433.

[‡] *Ibid.*, xiv. p. 612.

Its composition, making allowance for the ash (0·2 to 0·5 per cent.), was:—Carbon, 50·5 to 51·3; hydrogen, 6·4 to 7·0; nitrogen, 16·5 to 17·1; and sulphur, 1·1 per cent.

Its molecular weight determined in an aqueous solution by Raoult's method varied from 587 to 714.

For the composition of various albumoses and other proteoses prepared by Kühne, Chittenden, and their co-workers, see page 181.

Peptones.—Schrötter * controverts the generally accepted views as to the formation of albumoses as an intermediate stage in the production of peptones by enzymes. In his opinion both albumoses and peptones are precipitated by saturation with ammonium sulphate, but the former may be readily distinguished by their higher molecular weight, larger percentage of nitrogen, and by the fact that they contain sulphur, which peptones do not.

This view of Schrötter's illustrates the general want of agreement as to the nature of peptones, different chemists attaching that name to some certain group of the hydrolysed proteids, which they regard as more worthy of it than some other. It seems, however, most fitting to reserve the name for the most soluble of the derivatives, which are precipitated by strong alcohol, and are not removed by saturation with zinc or ammonium sulphate, although, even in the latter case, lower deuteroalbumoses may be grouped with the peptones.

Composition of Peptones.—The analyses by Chittenden † of peptones from different sources, given on p. 159, do not bear out Schrötter's theory that peptones differ from albumoses in not containing sulphur.

General Properties of Peptones.—Peptones are much more soluble than albumoses, or at least the higher albumoses (protoalbumoses), and require the addition of much alcohol to precipitate them from their solution. They are not coagulated by heat, and possess great diffusibility. They cannot be salted out by an addition of zinc or ammonium sulphate, a property which is usually regarded as the distinguishing feature between albumoses and peptones.

In the pure state a peptone is a hygroscopic amorphous powder, with a bitter taste. It rapidly absorbs moisture from the air and becomes resinous. In the anhydrous condition it dissolves in water with a hissing noise, and the evolution of a considerable amount of heat.

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* *Monatsheft. f. Chem.*, xiv. p. 612, and xvi. p. 609.

† *Medical Record*, 1894, pp. 486 and 546.

CHEMICAL COMPOSITION OF REPRESENTATIVE PEPTONES.

Peptone.	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
Amphopeptone from Blood Fibrin, .	48·75	7·21	16·26	0·77	27·01
Hemipeptone from Coagulated Egg Albumin, . .	49·38	6·81	15·07	1·10	27·64
Peptone from Hemp Seed, . . .	49·40	6·77	18·40	0·49	24·94
Antipeptone from Casein, . .	49·94	6·51	16·30	0·68	26·57
Antipeptone from Myosin,. . .	46·26	6·87	16·62	1·16	26·09

Many of the ordinary proteid precipitating reagents are not available for peptones. Thus, they are not precipitated by nitric acid with or without the addition of salt, by acetic acid with potassium ferrocyanide, or by an excess of picric acid. On the other hand, they are precipitated by chlorine or bromine, by tannin from a neutral solution, by phosphotungstic or phosphomolybdic acid, by uranium acetate, by mercuric chloride, and by absolute alcohol (*cf.* pages 164–176).

In the biuret reaction they resemble albumoses in giving purple colorations without warming, whilst albuminous substances give more of a violet colour which only becomes purple on applying heat.

Peptones can combine with either acids or alkalies to form salt-like compounds, and appear to form definite compounds with hydrochloric acid.

Hemipeptones and Antipeptones.—Of the peptones produced by the hydrolysis of albuminous substances or proteoses, part are capable of being further broken up by trypsin, with the formation of simpler substances, such as tyrosine or leucine. The *hemi-* group of the original molecule appears to contribute chiefly to these, and they were therefore termed *hemipeptones* by Kühne and Chittenden. For a similar reason the other portion of the peptones, which resist the action of the enzyme, received the name of *antipeptones.* Both kinds are grouped together under the name of *amphopeptones.*

Gelatin Peptones.—Under the influence of dilute acids, of digestive, and of bacterial enzymes, or of the continued action of boiling water, gelatin is converted into a much more soluble substance or substances known as gelatin peptone. The gelatin undergoes a change analogous to that which occurs in the digestion of simple albuminous substances, and the resulting product, or series of products, is no longer capable of gelatinising.

No method of separating these decomposition products of gelatin from ordinary peptones has yet been devised, although Salkowski has described a number of colour reactions which are said to distinguish the two classes of derivatives from one another (*cf.* page 162).

Reactions and Physical Properties of Proteids.

The Combination of Proteids with Hydrochloric Acid.—It is well known that in artificial digestion experiments the free hydrochloric acid gradually disappears, and that a further addition of it is required to carry on the process.

Cohnheim * has prepared albumoses and peptones according to Kühne and Chittenden's directions, and has determined the average amount of hydrochloric acid with which each kind can combine at a definite temperature.

At 40° C. he found that protoalbumoses (in 2·5 per cent. solution) combined with 4·32 per cent. of their weight of hydrochloric acid, deuteroalbumoses with 5·48 per cent., heteroalbumoses with 8·16 per cent., and antipeptones with 15·87 per cent. in the mean.

On varying the conditions of temperature and concentration there was a difference in the amounts of hydrochloric acid added, but there was invariably a constant relation in this respect between the three albumoses and the peptone employed.

The amount of hydrochloric acid absorbed can be determined by treating the albumose with a definite quantity of the acid in excess, salting out the compound with ammonium sulphate, and determining the residual acid in the filtrate. But this method is said not to be applicable in the case of deuteroalbumoses and antipeptones.

It is noticeable that the order in which these compounds can be arranged as regards hydrochloric acid absorption is not the same as the arrangement according to diffusibility, solubility, etc. Cohnheim suggests that probably albumoses can combine with hydrochloric acid in more ways than one, or, in other words, be di-basic.

Colour Reactions of Proteids.—There are numerous colour tests for proteid substances, but as many organic bodies, especially among the aromatic compounds, give similar colorations with the same reagents, they cannot be regarded as absolutely characteristic.

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* *Zeit. Biol.*, 1896, p. 489.

The Biuret Reaction.—On adding an alkali to the solution of a proteid, and then, drop by drop, a weak solution (2 per cent.) of copper sulphate, there is no precipitation of copper hydroxide, but the liquid becomes violet. Care must be taken to avoid an excess of copper salt, or the violet colour will be masked by the blue.

The name of the reaction is derived from the fact that biuret or allophanamide $[(CO)_2(NH_2)_2.NH]$ gives a similar purple or red colour under the same conditions. It is doubtful, however, whether the colour is produced by the same group in the molecules of biuret and of albuminous substances.

If a nickel salt be used instead of copper sulphate, the coloration will be yellow or orange.

According to Neumeister the reaction will detect one part of a proteid in 10,000 of water, while Hoffmeister gives the limit of sensibility as 1 in 12,000.

F. Klug * has based a quantitative method of estimating proteids on the spectroscopic examination of the liquid in the biuret test.

Millon's Reaction.—On boiling proteids with a solution of mercuric nitrate containing a little nitric acid, a red coloration or precipitate is obtained. This reaction is also given by aromatic compounds, such as tyrosine, in which only one atom of the benzene group is replaced by hydroxyl:—

$$C_6H_4\left\langle\begin{matrix}OH\\CH_2.CH(NH_2).COOH.\end{matrix}\right.$$

The reaction is much less pronounced with proteids than with tyrosine, but is probably due to the same group in the molecule.

Xanthoproteic Reaction.—On heating proteids with strong nitric acid, a yellow precipitate or colour is obtained from the formation of nitro derivatives. This becomes deep orange on the addition of ammonia in excess. The reaction is also given by many aromatic compounds.

Adamkiewicz's Reaction.—When albumin, in as dry a state as possible, is dissolved in glacial acetic acid, and half its volume of concentrated sulphuric acid added to the solution, a violet-red colour is produced either immediately or after boiling for some time.

Liebermann's Reaction.—When certain proteid substances are washed with alcohol and cold ether, and heated with concentrated hydrochloric acid (1·19 sp. gr.), they give a violet coloration.

Rimini † has shown that this is due to the presence of traces of vinyl alcohol in the ether.

* *Chem. Centralblatt*, 1893, ii. p. 499.
† *Gazz. Chim. Ital.*, 1899, p. 390.

Colour Reactions of Albumin and Gelatin Peptones.—Salkowski gives the following table of the colour reactions of albumin and gelatin in solution :—

	Albumin Peptone.	Gelatin.	Gelatin Peptone.
1 c.c. of Solution + (5 c.c. Acetic Acid + 5 c.c. Sulphuric Acid), . .	Violet.	Yellowish.	Yellowish.
Equal volumes of the Solution + concentrated Sulphuric Acid, . .	Dark brown.	Yellow.	Yellow.
Millon's reagent, . . .	Reddish.	Colourless.	Colourless.
5 c.c. of Solution + 1 c.c. of Nitric Acid (sp. gr. 1·2)—boil and add sodium hydroxide, . .	Dark orange.	Lemon-yellow.	Lemon-yellow.

Heat Coagulation of Proteids.—As many of the simple albuminous bodies coagulate at a different temperature, it is often possible to separate them by means of fractional coagulation. For this purpose Halliburton has devised the apparatus shown below.

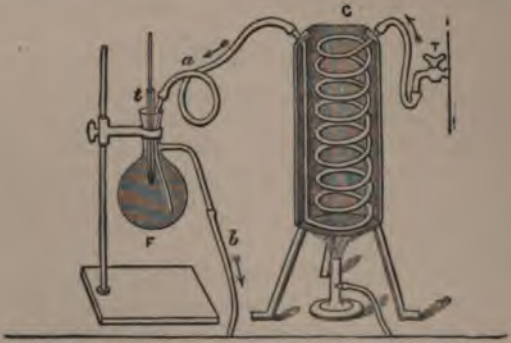

FIG. 18A.—Halliburton's apparatus. T, tap for water; C, copper vessel with spiral tube; *a* and *b*, inlet and outlet tubes; *t*, test-tube with fluid and thermometer.

The test-tube containing the solution of the proteids is kept in water at the given temperature for five minutes, while the degree of acidity is kept constant by the addition of dilute (2 per cent.) acetic acid, added from a burette, the right proportion to be added after neutrality being about 1 drop to each 3 c.c. of the liquid.

Thus, for example, from human blood serum there can be separated in this way fibrinogen, coagulating at 56° C.; serum globulin at 75° C.; and three kinds of serum albumin at 73° C., 77° C., and 85° C. respectively.

Of other important proteids, egg albumin coagulates at 72° to 73° C., myosin at 56° C., vitellin at 75° C., and hæmocyanin at 65° C.

J. H. Milroy * has studied the degree of coagulation which the albuminous substances of flesh undergo when heated at different temperatures. In each experiment the finely-divided flesh was heated in a beaker for an hour at temperatures ranging from 50° to 120° C., and the non-coagulated albuminous matter extracted with a solution of ammonium chloride (15 per cent.).

On extracting different kinds of flesh, without previous heating with this solution, the following amounts of proteids, calculated on 100 parts of the dry flesh, were extracted:—Fresh beef, 14·0 to 23·5; ham, 9·31; salt beef, 13·66; beef pickled in acetic acid, 5·87; calf's brain, 2·77. The difference between the coagulable albumin extracted from the non-heated flesh and from the same flesh heated at different temperatures gave the amount coagulated by the heat.

PROPORTION OF ALBUMINOUS SUBSTANCES COAGULATED BY HEAT.

Temperature. °C.	Fresh Beef.	Ham.	Salt Beef.	Beef pickled in Acetic Acid.	Calf's Brain.
50	45·95 to 55·10	45·87	63·55	67·13	51·99
60	64·37 ,, 74·47	54·25	84·04	88·44	80·15
70	90·66 ,, 91·01	95·28	95·32	100·0	84·12
80	99·11 ,, 100·0	99·18	100·00	100·0	90·26
90 to 120	100·0	100	100·00	100·0	100·0

In one experiment the fresh unheated flesh yielded to the ammonium chloride solution 22·77 per cent. of coagulable albuminous matters. On slightly roasting the same flesh the amount extracted from the interior was 13·08 and from the exterior 4·71 to 5·21 per cent., while the quantities obtained from the interior and exterior, after strongly roasting the meat, were 0·13 and 0·11 per cent. respectively.

Optical Rotation of Proteids.—All proteids rotate the beam of polarised light to the left. The specific rotatory powers of representatives of various groups are as follows:—

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* *Archiv Hyg.*, 1895, xxv. p. 154.

	[α] D.	Authority.
Egg Albumin, ...	−33·5°	Hoppe-Seyler.
Serum Albumin, ...	−56	,,
Syntonin from Egg Albumin,	−63·12	Haas.
Sodium Albuminate, . .	−55°	,,
Protoalbumoses, ... (various sources)	−71·40° to −79·05	Kühne and Chittenden.*
Deuteroalbumose, . . .	−79·11	,, ,,
Heteroalbumose, . . .	−68·65	,, ,,
Fibrinogen, ...	−43	Hermann.

Hoppe-Seyler † has devised a polarimetrical method of quantitatively estimating proteids, based on the difference in their rotatory power.

The Precipitation of Proteids by Various Reagents.

Precipitation of Proteids with Alcohol.—All proteids are insoluble in alcohol, and can be precipitated from their aqueous solutions by adding it in sufficient quantity. When albuminous bodies are precipitated by dilute alcohol they are apparently unaltered, but when the alcohol is concentrated and its action continued for some time, coagulation takes place, and the precipitate will not subsequently dissolve in water.

Alcoholic precipitation is often used as a means of separating the proteid constituents of meat extracts (*cf.* p. 199).

All such methods appear to depend on the fact that the further the hydrolysis of a given proteid has proceeded the more soluble become its products, and the greater the amount of alcohol required for their precipitation. Hence by varying the strengths of alcohol the proteid nitrogen might be subdivided in many different ways.

Precipitation of Proteids by Saturation of their Solutions with Salts.—It is often possible to effect a more or less complete separation of a proteid or group of proteids from its solution by saturating the liquid with a readily soluble salt, and this has been largely used in the quantitative analysis of proteid substances.

In such cases the precipitation is probably brought about merely by a withdrawal of the water required for the solution of the proteid, and is not due to the formation of a definite compound between the metallic salt and the proteid, as in the precipitations in Schjerning's method of analysis. The characteristics of the proteids thus 'salted out' remain unchanged. Certain proteids, such as peptones and some deuteroalbumoses, are soluble in concentrated solutions of ammonium or zinc sulphate.

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* *Zeit. Biol.*, xx. p. 11.

† *Virchow's Archiv*, xi. p. 547.

Saturation with Ammonium Sulphate.—For years this was the method generally employed for the separation of albumoses. The liquid was boiled and filtered to remove coagulable albuminous substances, and an excess of ammonium sulphate added to the filtrate when cold. The precipitate was collected, washed with a saturated solution of ammonium sulphate, boiled in water with barium carbonate to expel the ammoniacal nitrogen, and the residual nitrogen estimated by Kjeldahl's method.

Saturation with Zinc Sulphate.—Precipitation with ammonium sulphate suffers from the great drawback of the introduction of nitrogen during the precipitation, and the necessity of removing this before the proteid nitrogen of the precipitate can be estimated. To avoid this, Bömer made experiments with zinc sulphate as a precipitant, and found that it precipitated practically the same amount of proteid nitrogen. Subsequently, in conjunction with Baumann,* he made parallel experiments on the two methods of saturation to determine to what extent nitrogenous bases, amide bodies, and ammonia were precipitated in each case. The results showed that ammonium sulphate precipitates considerable quantities of tyrosine and leucine. With zinc sulphate, on the other hand, ammonium salts, asparagine, leucine, tyrosine, and kreatine, in the degree of concentration in which they occur in meat extracts, are not precipitated, or, at most, the amount of the precipitate is so small as to be negligible.

The precipitation is most complete after the addition of dilute sulphuric acid (1:4) in the proportion of 1 part to 50.

Magnesium Sulphate.—In Schjerning's opinion it is probable that all readily soluble sulphates would precipitate all albumins and albumoses if added to saturation in the presence of a little acetic acid. In his method of analysing proteid substances, magnesium sulphate is used in place of zinc or ammonium sulphates as the saturating agent (*cf.* p. 173).

Saturation with Sodium Chloride.—By means of this salt albumoses can be subdivided into two groups—primary proteoses, which are precipitated on saturating their neutral aqueous solution with sodium chloride, and secondary proteoses, which are only partially precipitated on the addition of nitric acid to the previously saturated solution (see pp. 156–157).

The Precipitation of Proteids by Metals in Relation to the Periodic Law.—Schjerning † has recently shown that salts of analogous metals precipitate practically the same amount of nitrogen from solutions of mixed proteids, whereas the metals of non-analogous series show a marked difference in this respect.

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* *Zeit. Unters. Nahr. Genussm.*, 1898, p. 106.

† *Zeit. anal. Chem.*, 1898, p. 73.

Parallel determinations were made on the lines of his general method (p. 171), with solutions of diastase, peptone, egg albumin, milk, and beer, and the following were the mean results obtained throughout the series :—

Nitrogen per cent. precipitated by										
Chlorides.		Acetates.		Sulphates.						
Tin. ($SnCl_2$)	Lead.	Iron. (Fe_2O_3)	Manganese. (Mn_2O_3)	Magnesium.	Zinc.	Cadmium.	Nickel.	Iron. (FeO)	Manganese. (MnO)	Sodium.
5·1	6·4	63·0	60·8	4· 8	40·0	38·2	35·5	37·5	44·3	43·4

The results obtained with chromium acetate were much too low, but Schjerning accounts for this on the ground that the complete analogy between chromium and iron is doubtful.

Although copper salts have some analogies with salts of the magnesium group, precipitation with copper sulphate gave very much lower results, either with or without saturation. For example :—

	Magnesium.	Copper.	Iron.
Beer, . . .	17·4	4·5	...
Egg Albumin, .	94·8	81·3	82·8

From this it is evident that the sulphates of copper and iron precipitate almost the same amounts of proteid nitrogen.

With the acetates of lead, copper, and mercury, which form a naturally ascending but not analogous series of metals, the following percentages of nitrogen were precipitated :—

	Lead Acetate.	Copper Acetate.		Mercuric Acetate.	
		Cold.	Boiling.	Cold.	Boiling.
Beer, . .	16·0	19·9	25·4	40·1	43·5
Wort, . .	20·8	20·8	24·3	47·0	46·3

With regard to the influence of the acid, it was found that for the same metal the acetate precipitates more nitrogen than the sulphate, and the sulphate more than the chloride. With the latter, the largest precipitation took place in the cold solution; with the other salts, on boiling.

The acetates of calcium and strontium precipitated respectively 84·4 and 85·4 per cent. of the nitrogen of egg albumin. Generally speaking, the precipitating power of metals appeared to increase with their atomic weight in the analogous series.

The salts of noble metals are unsuitable as precipitants, chiefly on account of the readiness with which they are reduced to the metallic state.

Schjerning gives the following summary with regard to the suitability of metallic salts as precipitating agents.

1. The sulphates and chlorides precipitate at most true albumin, and that often incompletely.

2. The acetates of the magnesium group, and of the extended magnesium group, precipitate only true albumins. The precipitating power appears to rise with the atomic weight.

3. The acetate of lead and its analogues (?) precipitate all proteids up to the albumoses.

4. The acetates of the analogous oxides Fe_2O_3 and Mn_2O_3 precipitate all proteids up to the real peptones.

5. Uranium acetate and phosphotungstic acid precipitate all proteids, being examples of analogous metals, though of different salts. Uranium acetate can also precipitate some of the ammoniacal nitrogen in the presence of phosphoric acid, whilst phosphotungstic acid precipitates the whole of it.

6. Mercuric chloride precipitates all the proteids up to the albumoses, or the same amount of nitrogen as lead acetate. Mercuric acetate precipitates all the proteids, and, in addition, more or less of the amide nitrogen.

Precipitation of Proteids with Phosphotungstic Acid.—This reagent is widely employed as a general precipitant for all proteid substances, but the separation is not sharp, and the further the hydrolysis of an albuminous substance has been carried, the less complete is the precipitation. Peptones are only incompletely precipitated, while, on the other hand, certain flesh bases, such as kreatine and kreatinine, are completely precipitated.

Mallett * classifies the proteid and amide bodies of commonly occurring food substances into three groups as regards their behaviour with phosphotungstic acid.

1. Those which, even in fairly strong solution, give no precipitate, *e.g.*, leucine, asparagine, aspartic acid, and tyrosine.

2. Those which are precipitated in strong solutions, the pre-

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* Abst. *Analyst*, 1898, p. 329.

cipitate dissolving on heating and reappearing on cooling, *e.g.*, glutamine, kreatine, kreatinine, hypoxanthine, carnine, and urea. Peptone precipitates coagulate, and partially dissolve on heating.

3. Those which are precipitated, the precipitate not being sensibly dissolved on heating, *e.g.*, egg albumin, fibrin, casein, legumin, globulin, vitellin, myosin, syntonin, hæmoglobin, albumose, gelatin, and chondrin.

The precipitates given by certain amide bodies are soluble in hot water to the following extent:—Betaine, 1 in 71 parts at 98·2° C.; kreatine, 1 in 107 parts at 98·1° C.; kreatinine, 1 in 222 at 97·9° C.; hypoxanthine, 1 : 98 at 97·6° C.; and carnine, 1 in 132 at 98·4° C.

Methods of Preparing and Using the Reagent.—(1.) From 5 to 10 grammes of phosphotungstic acid are dissolved in 100 c.c. of 2·5 per cent. hydrochloric acid (Mallet).

(2.) 120 grammes of sodium phosphate and 200 grammes of sodium tungstate are dissolved in water and the solution made up to a litre. The solution of proteid substance is mixed with dilute sulphuric acid (1 : 1) and the above solution in equal quantities at a temperature of from 60° to 65° C. After standing for twenty-four hours, the precipitate is collected, washed with sulphuric acid (1 : 2), and the nitrogen it contains estimated by Kjeldahl's method.

Precipitation of Proteids by Halogens.—*Chlorine Precipitation.*—Proteids combine with the halogens, forming insoluble substances. Although this fact was pointed out years ago by Mulder, it had been lost sight of until Rideal and Stewart* described a method of estimating proteids by precipitating them from their solutions with chlorine.

In their method, a current of chlorine is passed through the solution, which should contain not more than 0·2 per cent. of proteids, until the precipitate becomes granular and frothing ceases. After standing, preferably for some hours, the liquid is filtered through a hardened Schleicher and Schüll's filter paper, which has been previously weighed. The precipitate is washed with cold water, dried as far as possible in warm air, and finally, *in vacuo*, over sulphuric acid. The weight of the chlorine precipitate, multiplied by 0·78, gives the amount of proteid present.

In the test experiments described in the original paper, a determination of the nitrogen in the dried precipitate, and multiplication of the results by 5·5 in the case of gelatin, and by 6·33 in the case of the other proteids examined, gave satisfactory results in most instances.

It was found that meat bases, such as kreatinine, were not precipitated by chlorine.

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* *Analyst*, 1897, pp. 228–235.

Bromine Precipitation.—Some experiments were also made by Rideal and Stewart with bromine as the precipitant, and A. H. Allen * suggested the determination of nitrogen in the precipitate without previous drying.

The following simplified method was subsequently worked out by Allen and Searle † on these lines:—

The solution containing about 1 gramme of the proteid is diluted to 100 c.c., and rendered distinctly acid by the addition of dilute hydrochloric acid. A considerable excess of bromine water is then added, and the liquid stirred for some time. The precipitate is allowed to settle, the supernatant liquid decanted through an asbestos filter, the precipitate washed with cold water, and if necessary with bromine water, or sodium sulphate solution, and the nitrogen it contains determined by Kjeldahl's method, and calculated into proteid by the factor 6·33 (or 5·5 for gelatin).

Solutions of kreatinine, asparagine, and aspartic acid gave no precipitate with bromine under these conditions, and the precipitate given by 'meat extractives' extracted from fresh beef with water contained only a very inconsiderable amount of nitrogen.

Some of the principal results thus obtained were—

Substance.	Nitrogen per cent.		Nitrogen Multiplied by Factor.		Factor Employed.
	Total in Original Substance.	Precipitated by Bromine.	Total in Original Substance.	Precipitated by Bromine.	
Commercial Gelatin, .	14·10	14·00	77·5	77·0	5·5
Gelatin Peptone, . .	14·10	13·90	77·5	76·5	
Commercial Scale Albumin, ...	8·80	8·72	55·8	55·2	6·33
Syntonin from Scale Albumin, ...	9·86	9·60—9·76	62·41	60·77—61·78	
Digested Scale Albumin,	8·89	8·81	56·3	55·8	
Fresh White of Egg, .	1·89	1·88	11·96	11·90	
Syntonin from White of Egg, ...	1·89	1·89	11·96	11·96	
Peptone from White of Egg, ...	0·70	0·69	4·43	4·37	
Beef Extractives, . .	0·33	0·004	2·11	0·03	

There can be no question as to the value of halogen precipitation, since it has been shown, both by Rideal and by Allen, that meat bases are not precipitated (or if so the precipitates are soluble in dilute acid). Thus we have a means of exactly separating them from albuminous and gelatinous compounds, which has long been a want in the analysis of meat extracts and similar preparations.

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* *Analyst*, 1897, p. 233.

† *Ibid.*, 1897, p. 259.

Notes on Schjerning's Reagents.—*Uranium Acetate.*—Kowalewsky [*] showed that proteids were precipitated by uranium acetate, but that the precipitates were somewhat soluble in water. Albumins and globulins, and, according to Schjerning, albumoses and peptones, but not amido-compounds, such as asparagine and leucine, are precipitated. The precipitation may be made at the ordinary temperature, but must always be from a neutral or slightly acid solution (see also pp. 173 and 205). The chief precaution is to have the uranium solution quite clear and free from basic compounds. Schjerning states that if phosphoric acid be present in the solution in a larger proportion than the proteid, the ammoniacal nitrogen, and possibly a very little of the amide nitrogen present, are precipitated.

Tin Chloride was first proposed as a reagent for proteid precipitation by Drechsel [†] and by Siegfried.[†] It precipitates about 90 per cent. of the albuminous substances in white of egg, and in Schjerning's method of analysis (p. 171) all proteids precipitated by it are termed albumin I.

Lead Acetate.—Berzelius [‡] was the first to point out that albumin is quantitatively precipitated by basic lead acetate, but only partially by the normal salt. According to Schjerning it precipitates albumins, and proteid compounds not far removed from nucleïns (denucleïns).

Ferric Acetate [§] was proposed by Schmidt-Mulheim for the precipitation of albuminous substances and pro-peptones (proteoses). Schjerning found that it precipitated albumins, albumoses, and 'denucleïns.'

Stutzer's Copper Hydroxide Reagent.—This is prepared by dissolving 100 grammes of copper sulphate in 5 litres of water, adding 2·5 grammes of glycerin, then a dilute solution of sodium hydroxide until the reaction is alkaline, and filtering. The precipitate is thoroughly mixed with water containing 5 grammes of glycerin per litre, and decanted and filtered, until all alkali has been removed. The residue is triturated with a litre of water containing 10 per cent. of glycerin, until a mud is obtained which can be drawn up into a pipette. It is kept in a well-closed flask in the dark.

[*] *Zeit. anal. Chem.*, xxiv. p. 551.
[†] *Berichte*, xxiii. p. 3096 and xxiv. p. 418.
[‡] *Lehrbuch der Chem.*, ix. p. 43.
[§] *Zeit. anal. Chem.*, xix. p. 127.

This was said to precipitate albumoses, but not peptones or gelatin. But Rideal and Stewart * have shown that a considerable proportion of gelatin is precipitated, and that this is probably not due to any hydrolysis of the latter in the course of manufacture. They consider that the reagent cannot be relied upon to effect a separation of albumoses from gelatin, and this is borne out by their results, in which the amount of nitrogen precipitated by Stutzer's reagent is only about half that contained in the albumoses obtained by saturating the solution with ammonium sulphate.

	Nelson's No. 1 Gelatin.	Coignet's Extra Gelatin.	Swiss Gold Leaf Gelatin.	Somatose.	Witte's Peptone.
Total Nitrogen.	13·8	14·61	14·33	13·72	14·67
Nitrogen in Ammonium Sulphate Precipitate,	13·69	13·63	13·92	12·44	10·13
Nitrogen in Stutzer's Precipitate, . .	4·09	5·91	4·59	6·64	4·52

Schjerning's Method of Analysing Proteid Solutions.—From the fact that metals of analogous series precipitated the same amount of nitrogen from solutions of different proteids, while the nitrogen precipitated by metals of non-analogous series is dissimilar, Schjerning came to the conclusion that the precipitates are compounds of the respective metals with definite proteids.

To these proteid substances he has given the following provisional names, as indicating to some extent their character. From comparison with the results obtained with malt extract he considers that there are two kinds of albumin in milk.

Precipitated by,		Contains the Proteids.	Precipitated by,		Contains the Proteids.
Tin chloride	$=a$	Albumin I.			
Lead acetate Mercuric chloride	$=b$	Albumin I. Albumin II. Denuclëin.	Uranium acetate	$=d$	Albumin I. Albumin II. Denuclëin. Albumose. Peptone.
Ferric acetate	$=c$	Albumin I. Albumin II. Denuclëin. Albumose.	Magnesium sulphate	$=e$	Albumin I. Albumin II. Albumose.

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* *Analyst*, 1897, xxii. p. 230.

From these five precipitations, the amount of the different proteids or groups of proteids can be calculated, since

		Precipitate
Albumins I.	=	a
,, II.	=	$b-[a+(c-e)]$
Denucleïns	=	$c-e$
Albumoses	=	$c-b$
Peptones	=	$d-c$

The reagents required are :—

1. A solution of tin chloride, prepared by dissolving 50 grammes of tin in a weighed flask containing a sufficient quantity of boiling concentrated hydrochloric acid, and a little platinic chloride. The solution is evaporated down to about 130 grammes, made up to a litre and filtered.
2. A solution of normal lead acetate, containing about 10 per cent. of the salt, and 10 to 12 drops of 45 per cent. acetic acid per litre.
3. A 5 per cent. solution of mercuric chloride.
4. Pure dry ferric acetate.
5. Dilute acetic acid, containing 15 c.c. of 45 per cent. acid in a litre.
6. A solution of pure uranium acetate (about 10 per cent.) free from ammonia.
7. Pure crystallised magnesium sulphate.
8. A solution of ordinary sodium phosphate, containing about 0·4 per cent. of the crystallised salt.
9. Calcium chloride solution (about 10 per cent.).

The solution containing the proteids is first diluted, so that 10 c.c. contain a quantity of total nitrogen corresponding to about 5 c.c. of decinormal acid. In some cases, when the solution contains little or no ash, the addition of mineral matter (solutions 8 and 9) is necessary. This point may be determined by a preliminary test :—If the number of c.c. of the proteid solution which correspond to about 10 c.c. of decinormal acid do not, on boiling, completely precipitate the iron from a solution of 0·8 gramme of ferric acetate dissolved in 40 c.c. of dilute acetic acid (reagent 5), and 50 to 100 c.c. of water, the precipitations with tin, lead, and iron must be preceded by the addition of reagents 8 or 9.

The Tin Chloride Precipitation.—About 5 c.c. of the tin chloride solution (reagent 1) are added to 25 c.c. of the proteid solution. After stirring well, the beaker is covered with a glass, and left for from 6 to 24 hours. The precipitate is then collected on a filter and washed with cold water. If the proteid solution be poor in ash, 10 c.c. of calcium chloride solution (reagent 9) are added before the tin chloride, and the precipitate washed with a cold 1 per cent. solution of calcium chloride.

The Lead Precipitation.—To 25 c.c. of the proteid solution is added a sufficient amount of lead acetate solution (reagent 2), the amount varying with different substances. Care must be taken to avoid an excess of the reagent, or part of the precipitate may be redissolved. After adding the reagent, the liquid is boiled, and the precipitate collected and washed with cold water. If the proteid solution contains little ash, sodium phosphate solution (No. 8) is added before boiling, in the proportion of about three volumes to each volume of lead acetate solution used. Since the lead precipitate is somewhat soluble in the precipitating reagent, a correction is necessary. This Schjerning has found experimentally to be about 0·15 c.c. of decinormal acid for each 100 c.c. of filtrate and washings.

The Mercuric Chloride Precipitation.—Five c.c. of the mercuric chloride solution (No. 3) are added to 25 c.c. of the proteid solution, the liquid allowed to stand for 4 to 24 hours at the ordinary temperature, the precipitate filtered off, and washed with a cold 0·5 per cent. solution of mercuric chloride, and the nitrogen it contains estimated by Kjeldahl's method. The amounts of proteids precipitated by lead acetate and by mercuric chloride are identical, but as the latter usually gives more satisfactory results, the lead precipitation need only be used in exceptional cases.

The Iron Precipitation.—Ferric acetate (0·8 gramme) is dissolved in 40 c.c. of dilute acetic acid (reagent 5) and 50 to 100 c.c. of water, the solution being heated to the boiling point. 20 c.c. of the proteid solution are then added, and the liquid again heated to boiling. The precipitate is filtered off and washed three or four times with boiling water. The filtrate should be quite clear, and if this is not the case, from 15 to 25 c.c. of the sodium phosphate solution (No. 8) should be added immediately after the second boiling, the liquid being meanwhile stirred and kept boiling. With practice the right amount of phosphate can be estimated. Twenty c.c. have no injurious effect if these directions be followed, and only in exceptional cases is it necessary to add greater quantities (at most 25 c.c.).

The Uranium Precipitation.—To 25 c.c. of the proteid solution are added 20 to 25 c.c. of reagent 6, the liquid heated to the boiling point with constant stirring, and allowed to stand over night in a dark place. The precipitate is then collected on a filter, and washed with a cold 1 to 2 per cent. solution of uranium acetate. The correction for the solubility of the precipitate corresponds to 0·10 c.c. of decinormal acid for each 100 c.c. of filtrate and washings.

The Magnesium Sulphate Precipitation.—Five or six drops of 45 per cent. acetic acid are added to 20 c.c. of the proteid solution, and the beaker placed in a water-bath kept at 33° to 36° C. From 18 to 20 grammes of finely powdered magnesium sulphate ($MgSO_4 + 7H_2O$) are added, with constant stirring, and the liquid allowed to stand for thirty minutes to an hour at the ordinary temperature, a stir being given from time to time. The precipitate is then filtered off, and washed with a cold saturated solution of magnesium sulphate containing 4 to 5 grammes of 45 per cent. acetic acid per litre.

Some of Schjerning's results of the analysis of solutions of different proteids are shown in the subjoined table, which is compiled from others given in his various papers on the subject. A minus sign indicates an error in the calculated results, and where it occurs there was none of the given proteid present.

	Egg Albumin.		Serum of Calf's Blood.	Witte's Peptone.		Liebig's Flesh Peptone.		Liebig's Meat Extract.
	1.	1.		1.	2.	1.	2.	
Albumin I.,	89·2	85·0	83·4	2·7	3·0	13·5	13·9	10·7
„ II.,	1·9	2·2	12·0	...	18·8	2·5	...	0·0
Denuclëin, .	1·2	1·6	2·9	8·0	12·2	8·5	9·2	10·2
Albumose, .	3·9	5·0	0·3	48·5*	25·4	32·1	33·2*	6·1
Peptone, .	2·7	1·2	-1·5	0·0	-3·5	-1·6	0·0	11·4

Precipitation of Proteids by Certain Alkaloid Reagents.—*Tannin.*—According to Almen a solution composed of 4 grammes of tannin, 8 c.c. of acetic acid (25 per cent.), and 190 c.c. of dilute alcohol (40 to 50 per cent.) gives a precipitate in solutions of albumin, albumoses, or peptones containing 1 part in 100,000, after standing for twenty-four hours. The precipitates are soluble in excess of the reagent.

Mallet makes use of tannin in his method of separating amide bodies from proteids (p. 167).

Mercuric Iodide with Potassium Iodide, added to a faintly acid solution of mixed proteids, gives a precipitate which, according to Neumeister, is as complete as that given by phosphotungstic acid.

Picric Acid in excess gives a precipitate even in very dilute solutions of albumoses. Peptones are not precipitated (König).

Phosphomolybdic acid is sometimes used in place of phosphotungstic acid.

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* Including Albumin II.

Mercuric chloride precipitates the same amount of proteid nitrogen as lead acetate (see p. 173).

Action of Formaldehyde on Proteids.—E. Beckmann[*] has found that albumin, albuminates, hemialbumoses, casein, gelatin, etc., combine with formaldehyde to form insoluble compounds, whilst peptones are not rendered insoluble by the treatment. The solution, containing about 1 gramme of gelatin or other proteid, is evaporated to dryness with five or six drops of formalin on the water-bath. The residue is moistened with one or two more drops of formalin, and the heat continued for an hour or more. It is then digested two or three times with water at 60° to 70° C. in order to remove trioxymethylene, and is dried at 100° C. until constant in weight. If necessary, both proteid solution and formalin must be previously neutralised with calcium carbonate, since free acid causes the compound to be more or less soluble.

Beckmann states that pure gelatin thus treated is rendered completely insoluble, as is also the gelatose obtained by heating an aqueous solution of gelatin for thirty to thirty-five hours on the water-bath. Gelatin peptone hydrochloride, however, remains completely soluble.

He gives the following table of his results obtained with other proteids, the ash being deducted in each case.

	Per cent.
Serum albumin (Merck), ...	92·2
Egg ,, ,, ...	94·0
Alkaline albuminate (Grübler), dissolved in dilute sodium carbonate solution, ...	104·9
Hemialbumose (Merck), ...	92·2
Do., purified by precipitation with ammonium sulphate, ...	97·8
Albumin peptone hydrochloride (Paal), . .	trace
Casein (Merck), ...	93·9
Diastase, ...	21·8
Tryptone, ...	nil

For the application of this method to the analyses of commercial peptones and meat extracts see p. 199.

C. Lepierre,[†] who has recently studied the nature of the changes brought about by formaldehyde on certain proteid substances, finds that the action is one of dehydration and condensation with the fixation of CH_2-groups.

1. *Protoalbumoses* are rendered insoluble, and the precipitate obtained does not dissolve in hot water, in a 10 per cent. solution of sodium chloride (exclusion of heteroalbumoses), or in sodium carbonate solution.

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[*] *Forschungs Berichte*, 1896, iii. p. 324.

[†] *Journ. Pharm. Chim.*, 1899, p. 449.

2. *Deuteroalbumoses* are not simple substances, but consist of a series of analogous bodies conveniently grouped together. Of these, the members of higher molecular weight, *i.e.*, those approaching most nearly in composition to the protoalbumoses, are rendered insoluble by the treatment with formaldehyde, whilst those of lower molecular weight, approaching the true peptones, are converted into protoalbumoses, and only after long-continued action of the reagent are the latter transformed into insoluble derivatives.

3. *True Peptones*, in like manner, are converted into substances of deuteroalbumose nature, and these in turn into protoalbumoses.

The precipitates of these different compounds are insoluble in cold or boiling water, but when heated in an autoclave for one or two hours at 110° C. are hydrated and rendered completely soluble, the solutions giving the characteristic reactions of the proteids from which the precipitates were derived.

The condensed compounds are capable of being digested by acid pepsin, although with less readiness than the proteids before the treatment with formaldehyde.

The Decomposition of Proteids.

Decomposition of Proteids by Sulphuric Acid.—Like many other complex nitrogenous substances, proteids are unstable, and can be readily broken down into simpler compounds. Thus albumin, for example, when heated with dilute sulphuric acid, is partially converted by hydrolysis into an insoluble substance (anti-albumid), and partially into soluble substances, consisting principally of albumoses and their further decomposition products.

This cleavage into two distinct kinds of product on hydrolysis is regarded as evidence of the compound nature of the original molecule of the proteid, the two components being termed the *hemi* and the *anti* groups.

Antialbumid, which was originally named *hemi-protein* by Schutzenberger, may be taken as typical of the *anti*-group. In the sulphuric acid hydrolysis of albumin it constitutes about one-half of the resulting products.

It is insoluble in dilute acid, but dissolves in dilute solutions of sodium carbonate. When treated with acid pepsin it undergoes but little change, but alkalin trypsin converts it into a soluble peptone, known as *antipeptone*.

The nature of the final products formed in pancreatic digestion serves to distinguish the hemi- from the anti-groups, inasmuch as the former yield much simpler final substances, being converted into tyrosine and other amide bodies, while in the case of antibodies the process of digestion ends with the formation of antialbumoses and antipeptones.

The composition of antialbumids derived from egg albumin and serum albumin is shown in the following analyses of Kühne and Chittenden [*] :—

	Egg Albumin.	Serum Albumin.	Antialbumid from Egg Albumin.	Antialbumid from Serum Albumin.
Carbon, . .	52·33	53·05	53·79	54·51
Hydrogen, .	6·98	6·85	7·08	7·27
Nitrogen, . .	15·84	16·04	14·55	14·31

Decomposition of Proteids by Super-heated Steam.—By heating a simple albuminous substance, such as albumin, in superheated steam, it is converted more or less into substances of an albumose nature. Chittenden and Meara [†] obtained two albumose-like substances by heating coagulated egg albumin in a sealed tube at 150°, while sulphur was simultaneously liberated.

Neumeister, [‡] in his experiments with fibrin, obtained two distinctive soluble derivatives—atmidalbumin (precipitated by sodium chloride) and atmidalbumose (ἀτμις = steam).

The proportion of the two products depends on the duration of heating; the longer the action of the superheated water is continued the greater being the proportion of atmidalbumose. A certain proportion of peptones and further decomposition products are also found.

E. Salkowski [§] heated weighed quantities of flesh (freed as completely as possible from fat and sinew) and of fibrin with water at about 130° C. in a small pressure boiler and analysed the resulting solutions.

In the four experiments the amounts of albuminous substance and water used were :—

1. 600 grammes of flesh with 2400 c.c. of water for 8 hours at 131° C.
2. ,, ,, ,, ,, 120° C.
3. 550 grammes of pressed blood fibrin ,, 133° C.
4. 450 ,, ,, ,, 2000 ,, 130° C.

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[*] *Zeit. f. Biol.*, xix. pp. 167 and 173.
[†] *Journ. Physiol.*, xv. p. 501.
[‡] *Zeit. Biol.*, xxvi. p. 57, and 1898, xxxvi. p. 420.
[§] *Ibid.*, 1897, p. 190.

And the composition of the resulting solutions was:—

	1.	2.	3.	4.
Dry Substance, grammes, . .	50·25	47·87	69·00	61·73
Organic Substance, grammes,	45·30	42·52	68·55	61·35
Inorganic ,, ,,	4·85	5·35	0·45	0·38
Ash of Total Solids, per cent.,	9·85	11·16	0·66	0·62
Nitrogen, ,, ,,	14·61	13·93	16·63	16·62
Nitrogen of Ash-Free Substance,	16·20	15·96	16·74	16·76
Sulphur ,, ,,	0·51	0·53	0·71	0·75
Ratio of Sulphur to Nitrogen, .	1 : 31·70	1 : 30·20	1 : 23·6	1 : 22

The amount of flesh and of fibrin dissolved by once heating were 38·7 and 58·15 per cent. respectively. And in the case of flesh, one-third of the proteid substances and two-thirds of the mineral passed into solution.

The ratio of sulphur to nitrogen was 1 : 31, whilst in the fresh flesh it was as 1 : 16·7, a fact which showed that sulphur was split off in the process.

Atmidalbumin and Atmidalbumoses.—Neumeister found the characteristic products of the action of steam on fibrin (atmidalbumin and atmidalbumose) to be very refractory to the action of pepsin and trypsin and putrefactive decomposition, but Salkowski observed no difference in their behaviour in this respect from ordinary proteids.

In Salkowski's opinion it is probable that atmidalbumose may be able to replace albumin in food, though he considers that the question cannot be decided by the results of experiments on lower animals.

Atmidalbumin appears to be intermediate in its properties between albuminous substances and primary albumoses, and in Neumeister's opinion it is probably albumin hydrated without decomposition. On treatment with sulphuric acid atmidalbumin and atmidalbumoses are hydrolysed to deutero-albumoses, from which it may be inferred that they have a greater molecular weight than the latter.

Chittenden* considers that notwithstanding the fact of their being soluble in water, atmidalbumoses are closely related to the antialbumid formed by the action of acids on proteids, and that, like it, they are derived from the *anti*-group of the molecule.

He gives the following analyses to illustrate the change which is brought about by the action of superheated steam on coagulated egg albumin, together with an analysis of antialbumid for the purpose of comparison:—

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* *Med. Record*, 1894, p. 452.

	Coagulated Egg Albumin.	Atmidalbumose precipitated by sodium chloride.	Atmidalbumose precipitated by sodium chloride+acid.	Antialbumid.
Carbon, .	52·33	55·13	55·04	53·79
Hydrogen, .	6·98	6·93	6·89	7·08
Nitrogen, .	15·84	14·28	14·17	14·55
Sulphur, .	1·81	1·66	...	...
Oxygen, .	23·04	22·00	...	...

Decomposition of Proteids by Proteolytic Enzymes.

I. **Gastric or Peptic Digestion.**—The enzyme, pepsin, which is contained in the gastric juice acts upon native proteids in the presence of hydrochloric acid, breaking them down into simpler substances. In this process the tendency of the original molecule to cleavage into hemi- and anti-groups is observed, though not to the same extent as in the decomposition brought about by sulphuric acid.

In peptic digestion the albuminous substance (albumin, for example) is first converted into acid albumin or syntonin, which is then split up into primary proteoses or groups of proteoses, collectively called (in the case of albumin) amphoalbumoses. These consist of protoalbumoses and heteroalbumoses, to the former of which the hemi-groups of the original proteid molecule chiefly contribute, while the latter are mainly derived from the anti-group.

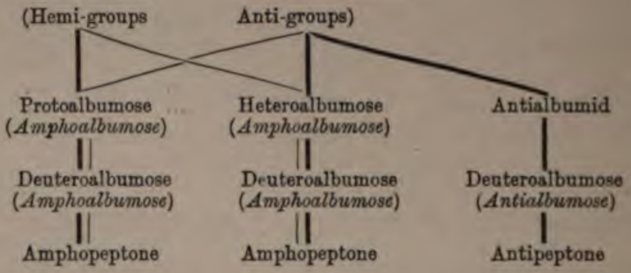
By the continued action of the enzyme the primary proteoses are further broken down with the formation of a mixture of secondary proteoses (amphoalbumoses). These are termed deutero-albumoses, and, like the primary albumoses, contain representatives derived from the hemi- and the anti-groups.

Finally there results a mixture of hemi- and anti-peptones, the former of which are characterised by being readily converted into simpler substances like tyrosine on treatment with alkaline trypsin, while the latter remain unchanged.

At different stages of the digestion a certain number of anti-groups of the compound may be split off to form substances of which antialbumid may be taken as the type, and this body, when the digestion is very active, is partially converted into deuteroalbumoses and antipeptones.

Neumeister * illustrates these changes by the following scheme, in which the relative proportion of hemi- or anti-groups in the hydrolysed derivatives is indicated by darker or fainter lines :—

ALBUMIN MOLECULE.



Owing to the want of accurate knowledge as to the relative size of the molecule, it has not yet been determined with certainty how many molecules of peptone are derived from each molecule of deuteroalbumose. Neumeister,† however, points out that according to Sabanjeff's determinations by Raoult's method, the molecular weight of protoalbumose from egg albumin is about 2400, and that of the peptone from the same source about 400. If this be correct, it would follow that six peptone molecules would be formed from each molecule of protoalbumose, and from the original albumin molecule (about 15,000) there would result some forty molecules of peptone. Hence thirty-four molecules of peptone would be derived from the heteroalbumose.

The hydrolysis of other proteids by pepsin gives products analogous to those yielded by albumin. Thus, fibrin yields protofibrinose, heterofibrinose, deuterofibrinose, and amphopeptones; whilst the proteoses derived from the myosin of muscle are termed protomyosinose and deuteromyosinose.

The composition of some of the more important of these derivatives is shown in comparison with that of their parent proteids in the following analyses, given by Chittenden.‡

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* *Zeit. Biol.*, 1887, p. v. 391.

† *Lehrbuch der Phys. Chem.*, 1897, p. 231.

‡ *Medical Record*, 1894, p. 486.

PROTEOLYSIS OF COAGULATED EGG ALBUMIN.

	Parent Proteid.	Proto-albumose.	Hetero-albumose.	Deutero-albumose.	Hemi-peptone.
Carbon, .	52·33	51·44	52·06	51·19	49·38
Hydrogen, .	6·98	7·10	6·95	6·94	6·81
Nitrogen, .	15·84	16·18	15·55	15·75	15·07
Sulphur, .	1·81	2·00	1·63	2·02	1·10
Oxygen, .	23·04	23·28	23·81	24·08	27·64

PROTEOLYSIS OF BLOOD FIBRIN.

	Parent Proteid.	Proto-fibrinose.	Hetero-fibrinose.	Deutero-fibrinose.	Ampho-peptone.
Carbon, .	52·68	51·50	50·74	50·47	48·75
Hydrogen, .	6·83	6·80	6·72	6·81	7·21
Nitrogen, .	16·91	17·13	17·14	17·20	16·26
Sulphur, .	1·10	0·94	1·16	0·87	0·77
Oxygen, .	22·48	23·63	24·24	24·65	27·01

PROTEOLYSIS OF MYOSIN FROM MUSCLE.

	Parent Proteid.	Protomyosinose.	Deuteromyosinose.
Carbon, .	52·82	52·43	50·97
Hydrogen, .	7·11	7·17	7·42
Nitrogen, .	16·77	16·92	17·00
Sulphur, .	1·27	1·32	1·22
Oxygen, .	21·90	22·16	23·39

PROTEOLYSIS OF GELATIN.

	Parent Proteid.	Protogelatose.	Deuterogelatose.
Carbon, .	49·38	49·98	49·23
Hydrogen, .	6·81	6·78	6·84
Nitrogen, .	17·97	17·86	17·40
Sulphur, .	0·71	0·52	0·51
Oxygen, .	25·13	24·86	26·02

PROTEOLYSIS OF ELASTIN.

	Parent Proteid.	Protoelastose.	Deuteroelastose.
Carbon, .	54·24	54·52	53·11
Hydrogen, .	7·27	7·01	7·08
Nitrogen, .	16·70	16·96	16·85
Sulphur, Oxygen,	21·79	21·51	22·96

It is interesting to note from these results that, in general, the proportion of carbon falls as the hydrolysis proceeds. In the case of gelatin, however, there is no marked difference in the composition of the parent proteid and its derivatives, while protoelastose contains slightly more carbon than the elastin from which it was derived.

This is another instance of the many particulars in which these two albuminoids differ from albuminous substances.

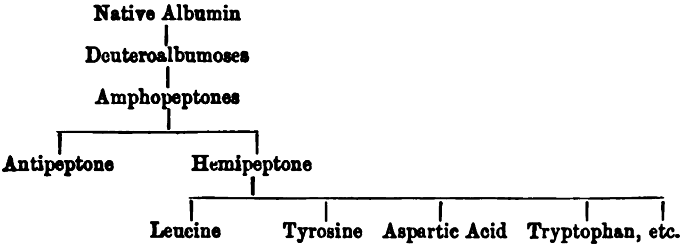
It is not possible to artificially convert the whole of a given proteid into peptones, as some intermediate products resisting the action of the pepsin are invariably left. Thus, in Chittenden's experiments the largest yield was about 60 per cent., and the average below 50 per cent. The process, which is at first rapid, soon becomes slow, possibly owing to the interference of the products, which in natural digestion may be removed as soon as formed. Experiments were therefore made in which the digestion was carried out in a parchment dialyser, so as to imitate more closely the natural process by removing the soluble products. In no instance, however, was there any marked increase in the yield.

From the results of his own experiments and those of others on the living subject, in which, after the digestion of egg albumin for forty-five minutes, it was found that more albumose than peptone had been formed, Chittenden considers that complete peptonisation is not brought about either in natural or artificial peptic digestion, and that the function of the process is to prepare the way for the profounder changes caused by pancreatic digestion.

II. **Pancreatic Digestion.**—The action of the enzyme (trypsin) of the pancreas on proteids is much more pronounced than that of pepsin, from which it also differs in being most active in a slightly alkaline medium (0·5 to 1 per cent. sodium carbonate). A certain proportion of free acid is required by pepsin, but the action of trypsin is arrested by the presence of much hydrochloric acid.

By the action of alkaline trypsin natural proteids are rapidly converted into peptones. Primary proteoses are seldom found, the proteid being apparently first converted into deuteroproteoses.

Neumeister* gives the following scheme illustrating the changes which albuminous substances undergo in trypsin digestion :—



Theoretically, it should be possible to convert the whole of the hemipeptone into crystalline substances, such as leucine, etc., but in practice Chittenden † found that upwards of 50 per cent. remained, even after long-continued digestion.

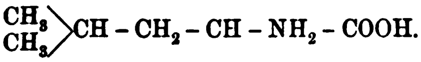
At various stages of the digestion, antialbumids may be split off, but this appears to happen less in natural digestion than in artificial digestion carried out in a flask. Where it occurs in the system it must represent a loss of nutriment, since antialbumids are very resistant to further decomposition.

In artificial digestion experiments they separate from the liquid in the flask in the form of a gelatinous mass. The amount depends on such conditions as the nature of the original proteid and the strength of the solution of trypsin, but under favourable circumstances may be as much as 25 per cent.

Chittenden gives the following analysis of myosin antialbumid formed in the trypsin digestion of myosin from muscular tissue:—Carbon, 57·48; hydrogen, 7·67; nitrogen, 13·94; sulphur, 1·32; and oxygen, 19·59.

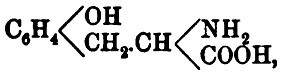
The most important of the characteristic crystalline products of trypsin proteolysis are :

Leucine or amido-caproic acid,



$$\begin{matrix} CH_3 \\ CH_3 \end{matrix} > CH - CH_2 - CH - NH_2 - COOH.$$

Tyrosine or para-hydroxy-phenyl-a-amido-propionic acid,



$$C_6H_4 < \begin{matrix} OH \\ CH_2.CH < \begin{matrix} NH_2 \\ COOH, \end{matrix} \end{matrix}$$

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* *Lehrbuch Phys. Chem.*, p. 247.

† *Medical Record*, 1894, p. 546.

which are representative of two distinct groups (fatty acid and aromatic) in the original proteid molecule. Both are formed in considerable quantity in trypsin digestion. Thus, for example, Kühne found in a typical experiment 9·1 per cent. of the former and 3·8 per cent. of the latter. They are formed in much larger proportion in artificial digestion than in the natural process in the body. Other crystalline products which have been isolated are, lysine or di-amido-caproic acid ($C_6H_{14}N_2O_2$); lysatine ($C_6H_{13}N_3O_2$), a member of the kreatinine group (p. 8); aspartic acid or amido-succinic acid ($C_4H_7O_2$); and glutamic acid ($C_5H_9O_4$).

Tryptophan or Protein-chromogen is a curious product of trypsin digestion, and is also formed in the decomposition of proteids by other agents. It combines with chlorine and bromine, forming with either a brilliantly coloured compound, which, in the case of the latter halogen, has been called the 'bromine body.' The analysis of an impure preparation by Stadelmann gave the following results:—Carbon, 49·00; hydrogen, 5·28; nitrogen, 10·99; sulphur, 3·77; oxygen, 110·1; and bromine, 19·95 per cent.

Chittenden * calculates the composition of the tryptophan to be:—Carbon, 61·02; hydrogen, 6·89; nitrogen, 13·68; sulphur, 4·69; and oxygen, 13·71 per cent. In his opinion the evidence all points to its being a synthetical compound, resulting from the union of two or more of the decomposition products of the original proteid, and containing the sulphur which is liberated from the hemi-peptones in their conversion into crystalline products.

Identification of the Products of the Digestion of Fibrin by Alkaline Trypsin.—Any unaltered fibrin is removed by slightly acidifying the alkaline liquid with acetic acid and boiling. The neutral filtrate is concentrated on the water-bath, and a portion tested for deuteroalbumoses by diluting it with an equal volume of saturated salt solution and adding acetic acid.

Another portion is saturated with zinc sulphate, and the filtrate tested for peptones by the biuret reaction. When present these are precipitated by adding bromine, or, with less exactness, by tannin.

When the digestion has proceeded for several days, the solution contains neither coagulable albumin nor albumoses. In such cases the neutralised solution on concentration deposits most of the tyrosin as a crystalline mass. The filtrate, when evaporated further and allowed to stand, yields a further deposit of tyrosine occasionally mixed with leucine, the former crystallising in highly refractive needles, the latter in globular masses.

* *Medical Record* 1894, p. 548.

These amido-acids can also be separated by extracting their solution with boiling alcohol, which dissolves only the leucine. The residual tyrosine is purified by dissolving it in warm ammonium hydroxide, reprecipitating it by neutralisation and washing the precipitate with water.

Trypsin Digestion of Gelatin.—The products formed in the pancreatic digestion of gelatin are analogous to these formed in the peptic digestion—viz., protogelatoses, deuterogelatoses, and gelatin peptones. But amido-acids do not appear to be produced, provided all bacterial action be prevented. Native collagene, the parent substance of gelatin, is not acted upon by alkaline trypsin.

III. **Papayotin Digestion.**—The enzymes secreted by insectivorous plants are analogous to animal pepsin and trypsin, and cause a similar decomposition of proteid substances. That contained in the juice of the *papaw* tree, *Carica papaya*, of Java, is the best known. It is found in all parts of the plant, but is usually extracted by leaving the juice of the unripe fruit until the resins deposit, and precipitating the papayotin from the filtrate with alcohol. It differs from pepsin in not acting in hydrochloric acid solution, and from trypsin in being less thorough in its action. In a 0·2 per cent. alkaline solution it is said to hydrolyse from seventy to eighty times its weight of fibrin in a few hours.

It has been stated that the conversion effected by papayotin does not proceed further than the formation of various albumoses, but Chittenden * has found that true peptones are formed. Neumeister has met with substances of the nature of atmidalbumin and atmidalbumose. Cibil's and Antweiler's flesh peptones are both said to be manufactured by means of papayotin.

Decomposition of Proteids by Bacteria.—The action of many of the species of bacteria on proteid substances is similar to that of the enzymes, and it is not improbable that such enzymes are present in the bacterial cells. According to the conditions of the hydrolysis, albumoses, peptones, phenol-compounds, and an immense number of simpler substances, among which may be found one or more of the ptomaines (p. 223), are produced.

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* *Amer. Jour. Physiol.*, 1898, i. p. 25.

CHAPTER IX.

MEAT EXTRACTS AND FLESH PEPTONES.

Of recent years the composition, food value, and methods of examining extracts of meat and similar substances have received a considerable amount of attention, which is not surprising considering the number of new preparations continually put upon the market, and the exaggerated statements of efficacy put forward by the vendors of some of these.

Manufacture of Meat Extract.—Although the name of Liebig is inseparably connected with a certain class of these products, the great German chemist was not the first who proposed to concentrate the soluble part of flesh, although he was the first to show how the suggestion might be carried out with commercial success.

The first experiments were made in Munich under the direction of von Pettenkofer in 1850–1852, and eventually the manufacture was commenced on a large scale at Fray Bentos in Uruguay.

The method originally proposed by Liebig consisted of soaking the finely divided flesh in eight times its weight of cold water, filtering the liquid from the insoluble fibre, heating it to coagulate the dissolved albumin, filtering this off, and concentrating the filtrate by evaporation to a syrup. In practice, however, it was found necessary to employ a higher temperature for the extraction; the flesh, in a fine state of division, was mixed with the requisite amount of cold water (free from calcium sulphate), and the mixture gradually heated to 180° F.

Since from 30 to 32 lbs. of lean meat are required to produce 1 lb. of meat extract (freed from albumin and fat), and the amount of lean meat in a cow only amounts to some 300 to 350 lbs., it is obvious that meat extract could not be profitably manufactured in Europe.

The residue left after the extraction of the soluble matter is used as manure (*Fleischknochenmehl*), and, when mixed with salts and other substances, has been made into various articles of human food (*cf.* p. 106).

The manufacture of 'Liebig's Extract of Meat' has been decided by the High Court of Justice to be public property, and there are now several firms, in addition to the original Liebig's Company, which sell a preparation under that name.

Beef Tea.—In ordinary beef tea there is generally a fairly large proportion of gelatin, owing to the boiling water acting on the collagene and rendering a large proportion of it soluble. The mineral salts and meat bases will be even more completely extracted than in the manufacture of commercial meat extracts, and the higher temperature of the water will also cause a large amount of the fibrin to be hydrolysed with the formation of albumoses, and possibly in some cases peptones. Analyses by the alcohol method of various kinds of concentrated beef tea are given on page p. 200.

Physiological Value of Extract of Meat.—*Water.*—In judging of the comparative value of different kinds of meat extracts, the degree of concentration is obviously of primary importance, for the less the quantity of water left, the greater will be the proportion of solid constituents.

Food Value.—Liebig expressly stated that his extract of meat was to be regarded as a stimulant, like tea or coffee, and not as a food, and his view is in the main confirmed by the experiments of later chemists. Thus, to mention one only of the more recent statements, C. Voit [*] asserts from the results of his experiments that extract of meat is practically useless as a food.

Albumin.—For this reason albumin is removed from the extract, since although it has a definite food value the quantity is too small to be of service, and at the same time the value of the preparation as a stimulant is reduced. An addition of albumin is made to certain preparations, but can scarcely be regarded as of any material service.

Meat Bases.—The meat bases are undoubtedly the most important constituents of meat extract. They have a stimulating action on the heart, increase the secretion of the saliva, and aid the digestive processes.

As is mentioned in the description of leucomaines (p. 218), some of the flesh bases act as poisonous alkaloids when taken in large quantity, and to this must probably be attributed the injurious effects which result from taking beef tea or meat extract in too great excess. [†] They appear to be of no value as foods, for Rubner [‡] found that kreatinine does not even act like gelatin in saving albumin.

Added Meat Fibre.—In several preparations an addition of flesh powder (with or without albumin) is made with the object of giving them some definite food value. As this amounts to at most some 8 or 10 per cent., it is obvious that a large quantity of the substance would be required to obtain as much unaltered proteid as is contained in an egg. On the other hand, it has been pointed out that there is nothing to show that flesh powder suspended in meat extract is more digestible than ordinary flesh in the same fine state of division, whilst the amount of flesh bases, the principal stimulating agents, is correspondingly reduced.

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[*] *Munich Med. Woch.*, 1897, 44, p. 219.

[†] See Aitken's *Animal Alkaloids*, p. 45.

[‡] *Zeit. Biol.*, 1884, p. 265.

Gelatin.—In the commercial process of manufacturing meat extract care is taken to extract the flesh at as low a temperature as practicable in order to prevent the formation of gelatin from the collagene. In ordinary household beef tea, where this precaution is not taken, gelatin forms a considerable proportion of the final product.

Although gelatin is not altogether valueless, its food value is of a very different order to that of albumin, and if the latter should be removed from meat extracts as interfering with their stimulating properties, much more so should the former.

The function of gelatin in the system is to save the albuminous substances which would otherwise be oxidised with the formation of heat, but it is quite incapable of replacing the nitrogen daily lost by the disintegration of the cells of the body. For instance, Voit * found that a hungry dog lost 5·3 grammes of nitrogen per day, but by giving it gelatin the daily loss sank to 2·1 grammes. This latter quantity represented the loss from the organic nitrogen of the cell decomposition; and it was found that by adding that amount of albuminous nitrogen to the gelatin, equilibrium was established.

Added Salt.—In analyses of these preparations it is usual to calculate the whole of the chloride into sodium chloride, although the greatest proportion of the naturally occurring chlorides in flesh consists of potassium chloride. In order to calculate the amount of added salt, Allen † makes an allowance of 0·06 per cent. of chlorine as sodium chloride for each unit per cent. of solid matter present, and deducts this from the total chlorine as sodium chloride found in the extract.

Liebig stated that no addition of common salt was required, and it is manifest that, like added meat fibre, such an addition must lessen the proportion of meat bases.

Fluid Beef and 'Peptones.'

Various methods of acting upon meat fibre, so as to convert it into soluble products, have been employed in the manufacture of fluid beef and the so-called peptones, the intention in each case being to have not only the flesh bases and other extractives of the meat, but also the nutritive part.

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* *Zeit. Biol.*, 1884, p. 284.

† *Commercial Organic Analysis*, iv. p. 303.

The Action of Superheated Steam.—By the hydrolysing action of superheated steam, fibrin is converted into substances of an albumose nature. This method was first described by Wöhler, who obtained a brown liquid on heating muscular fibre with water for two to three hours in a sealed tube. The nature of the products thus formed (atmidalbumoses, etc.) and the changes caused in flesh are described on pages 177–178.

Koch's and Kemmerich's 'Peptones.'—These are said to be manufactured by modifications of this process, the collagene being first removed from the flesh as completely as possible. Analyses of these are given on pages 198 and 203.

Somatose.—This is a preparation which has met with a considerable amount of success in Germany. It is said to be manufactured by a steam process, and is classified by Denaeyer with such preparations. According to the latter authority* it does not contain true peptones but has a large proportion alkali-albumin (albuminate).

According to an analysis by O. Hehner, somatose has the following composition:—Water, 14·16; fat, 0·41; total nitrogen, 11·54; proteid nitrogen, 10·88; albumoses, 62·13; peptones, 5·87; meat bases, 3·50 (factor 6·3); ash, 5·26; and difference 8·67 per cent.

A. R. Tankard,† using Allen's bromine method, found the composition of another sample to be:—Water, 14·25; total nitrogen, 10·78; proteid nitrogen, 9·94; alkali-albumin, 21·83; coagulable albumin, 3·40; albumoses, 33·96; peptones, 3·06; meat bases, 2·62 (factor 3·12); ash, 5·30; difference, 15·58.

As regards the food value of somatose, F. Massen ‡ states that it has a high nutritive value and can replace albumin in the animal system. When given to anæmic persons it is said to increase the number of red corpuscles and the amount of hæmoglobin.

Dr. Priebsch informed the author that in the military hospital in Vienna it has been found of considerable service in the case of patients who were unable to assimilate other food.

On the other hand, R. Neumann § states that these conclusions are not altogether borne out by the results of his experiments.

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* *La Composition des Peptones de Viande*, 1896, p. 5.

† Allen's *Commercial Organic Analysis*, iv. p. 384.

‡ *Zeit. Untersuch. Nahr. Genussm.*, 1898, p. 260.

§ *Munich Med. Woch.*, 1898, pp. 72–76 and 116–119.

'*Peptarnis.*'—This is a preparation manufactured by the Liebig's Extract of Meat Company, and is probably the same as Kemmerich's original peptone. The manufacturers state that its composition is:—Water, 28·95; gelatin, 3·92; albumin, 1·85; albumoses, 23·42; peptones, 23·06; meat bases, 8·94; fat, 0·18; sodium chloride and phosphates, 9·68 per cent. Total nitrogen, 9·95 per cent.

By precipitating the filtrate from the albumoses with bromine water, Allen found only 7·67 per cent. of peptones, and probably a large proportion of the nitrogen of the peptones in the manufacturers' analysis ought to be assigned to the flesh bases. (*Cf.* pp. 201–202.)

The Action of Pepsin.—Peptones are manufactured by the action of pepsin on finely divided flesh acidified with tartaric acid or hydrochloric acid.

According to Denaeyer,* the tartaric acid peptones are in the form of a white hygroscopic powder with a marked after-taste. Owing to the length of time required for the action of the pepsin (at least twenty-four hours) and the large excess of tartaric acid necessary the proportion of albumoses and peptones does not exceed 35 per cent. and further decomposition products are formed. By the use of hydrochloric acid (2 per cent.) instead of tartaric acid a higher yield of albumoses and peptones can be produced, up to 70 per cent. being precipitable by alcohol, although Denaeyer states that in practice the quantity rarely exceeds 60 per cent.

For the nature of the changes in proteids under the action of pepsin see pages 179–182.

The Action of Trypsin.—Numerous pancreatised products are in the market. They are prepared by the action of alkaline trypsin on flesh, blood fibrin, milk, etc. As a rule they contain more or less of the amido-decomposition products of the proteids such as tyrosin, leucin, etc., which have a bitter taste (*cf.* pp. 182 and 203). The peptones of Sanders Ezn and of E. Merck are said to be prepared by this process.

Papayotin Peptones.—Cibil's peptone in America and Antweiler's peptone in Germany are stated to be the products of the action of papayotin on flesh (*cf.* pp. 198 and 203). It is said that Antweiler's preparation is manufactured by removing the collagene from the flesh as completely as possible by boiling it with water, and then acting on the residual fibre with the juice of the plant.

Physiological Value of Fluid Beef and Flesh Peptones.—An enormous amount of controversy has taken place on the subject of the nutritive value of these semi-digested products, and conflicting evidence as to the results of experiments with various preparations is brought forward.

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* *La Composition des Peptones de Viande*, pp. 6–8.

Absorption of Proteids.—The view formerly accepted was that all proteids were converted in the intestine into peptones, and that the latter by reason of their more diffusible nature were readily absorbed into the blood. It is now known that the process of peptonisation is not necessary for absorption, and that, when it does occur, some further alteration of the albumoses and peptones formed must be brought about by the blood capillaries of the intestine wall before they can pass into the blood.

Absorption of Syntonin and Albuminates.—Many, if not most, of the albuminous substances can be directly absorbed as acid or alkali compounds, as was shown by the experiments of Voit and Bauer, who introduced syntonin from beef muscle and albuminate from egg albumin, into the small intestines of dogs, from which enzymes had been previously removed, and found that whilst only traces of albumoses and peptones could be detected at any given stage, the proteids were completely absorbed in one to four hours.

Injection of Albumoses and Peptones.—Moreover, albumoses and peptones are never found in the blood, and when introduced by way of a vein are promptly eliminated through the kidneys. If the quantity injected be large, symptoms of poisoning ensue similar to those caused by toxalbumoses. In most cases the coagulation of the blood is interfered with, although it is remarkable that *protoalbumose* and *antipeptone* do not have this effect (Kühne).

Food Value of Albumoses and Peptones.—Experiments have shown that certain albumoses and peptones are quite capable of replacing the daily loss of nitrogen arising from the decomposition of the cells, and establishing equilibrium, but in Neumeister's opinion* they have no more nutritive value than native albuminous substances for healthy persons, and he considers the point as more than doubtful in the case of invalids.

C. Voit,† too, found in his experiments that antipeptones acted like gelatin in sparing albumin, though they could not replace it. (*Cf.* p. 188.)

A further question arises as to the effect of the continued use of albumose and peptone preparations as substitutes for native albuminous substances. From the observations of Zuntz, of Gerlach, and of Pfeiffer with various substances of this nature, unpleasant after-effects frequently result.

In the present state of our knowledge, or rather want of knowledge, as to the changes which peptones and albumoses undergo before their absorption into the system, it seems somewhat premature to conclude from insufficient experimental data that the hydrolysed products of proteids, obtained by an artificial process outside the body, can universally replace natural albuminous substances or their digestion products formed under imperfectly known conditions.

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* *Lehrbuch Phys. Chem.*, p. 306.

† *Viertelj. Chem. Nahr. Genussm.*, 1897, p. 165.

The Analysis and Composition of Meat Extracts and Peptones.

The determination of water, mineral matter, total nitrogen, soluble albumin, gelatin, and added meat fibre are now usually made as described in Stutzer's method (p. 193), with slight modifications, and it is only in the differentiation of other kinds of soluble nitrogen that essential differences are found in the methods used by various chemists.

The author has made use of the following method in recent analyses of these preparations.

Syntonin is determined in the filtrate from the coagulated albumin by rendering the liquid slightly acid with acetic acid, adding potassium ferrocyanide, and heating. If any precipitate formed on the addition of the reagent does not redissolve, the liquid is exactly neutralised, with litmus as indicator, the precipitate filtered off, and its nitrogen determined by Kjeldahl's method, and multiplied by the factor 6·25.

Albumoses.—The filtrate from the syntonin, or, in its absence, from the coagulable albumin, is saturated with zinc sulphate as described on page 164. The precipitate is washed with a saturated solution of zinc sulphate, and the nitrogen it contains estimated in the usual manner and multiplied by the usual factor.

Peptones.—To an aliquot portion of the filtrate from the albumoses is added an excess of bromine water as recommended by Allen, the precipitate being collected and its nitrogen estimated as described on page 168.

Ammoniacal Nitrogen.—A second aliquot part of the zinc sulphate filtrate is distilled with barium carbonate.

Meat Bases and Amido-Compounds.—The total nitrogen in a third aliquot portion of the filtrate from the zinc sulphate precipitation is determined, and the difference between the result and the peptone and ammoniacal nitrogen previously determined may be taken as the nitrogen of the meat bases and other nitrogenous compounds.

Stutzer's factor (3·12) is most commonly used for calculating the nitrogen into meat bases, etc., but is only an approximation. Hehner prefers to use the factor 6·25 for all the nitrogenous constituents, since although this is much too high for the meat bases, it makes the non-nitrogenous extractives (determined by difference) considerably lower, and the two errors balancing one another to some extent, he considers that the results thus calculated represent more nearly the true composition of the preparation.

Stutzer's [*] **Method.**—i. *Estimation of Water, Ash, Sodium Chloride, and Total Nitrogen.*—From 5 to 7 grammes of a dry preparation, or from 20 to 25 grammes of fluid extract, are weighed into a thin tinfoil basin, dissolved in a little hot water, and ignited sand (freed from dust by means of a sieve) added in sufficient quantity to absorb the liquid. The basin and its contents are then dried until the weight is constant, the loss giving the water. The basin and the residue are subsequently used in the estimation of gelatin (see iv.).

Similar quantities of the preparations are taken for the determination of the ash, sodium chloride, and total nitrogen, all of which are carried out in the ordinary manner.

ii. *Nitrogen in the Form of Unaltered Proteids, Coagulable Albumin, and Flesh Powder.*—In order to detect the presence of meat fibre, the extract is treated with cold water and examined microscopically. If fibre be found the following method is adopted:—From 5 to 25 grammes of the preparation according to its state of dryness are repeatedly extracted with cold water, the insoluble matter collected on a filter, and the nitrogen it contains determined. This gives the nitrogen of the flesh powder with slight quantities of other unaltered proteids.

The filtrate is acidified with acetic acid, boiled, and filtered. The nitrogen of the insoluble portion is that present in the form of coagulable albumin.

When meat-fibre is absent, a weighed portion of the extract is treated with water and acetic acid, and the nitrogen of the insoluble portion (coagulable albumin) determined as before.

The filtrate may also be made up to a definite volume and the nitrogen determined in an aliquot portion. The difference between the result and that of the total nitrogen gives the amount present in the form of albumin.

iii. *Nitrogen in the Form of Ammonium Salts.*—From 5 to 25 grammes of the preparations are dissolved in water, barium carbonate added, and the ammoniacal nitrogen distilled into standard acid.

iv. *Gelatin Nitrogen.*—The residue of sand and extract left in the determination of the water in i. is ground in a mortar, the tinfoil cut into small strips, and the whole placed in a beaker where it is extracted with 100 c.c. of absolute alcohol, the supernatant liquid being removed each time by filtration through an asbestos filter.

The residue is now treated with a mixture of alcohol and ice-water, prepared by mixing in a large flask 100 grammes of alcohol with about 300 grammes of ice and adding sufficient water to bring the total weight up to one kilogramme. This flask and four beakers (*b*, *c*, *d*, and *e*) are placed in a bath filled with broken ice. The beaker *a*, containing the sand, peptone, etc., is also placed in the ice-bath, and 100 c.c. of the alcoholic ice-water poured into it, care being taken to keep the temperature of the mixture below +5° C. After the whole has been stirred with a glass rod for about two minutes, the supernatant liquid is poured into beaker *b*, a piece of ice being added at the same time. The extraction in beaker *a* is then repeated with a fresh portion of alcoholic ice-water, the liquid being decanted into beaker *c*; and this process is continued until the liquid above the sand is completely colourless.

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[*] *Zeit. anal. Chem.*, 1895, pp. 372 and 568.

In order to filter the extracts three asbestos filters are used. These consist of funnels about 7 cm. in diameter at the top, in each of which is placed a perforated porcelain disc covered with long-fibred asbestos. The first filter receives the liquid in beaker *a* and the insoluble residue excepting the sand. The contents of beaker *b* are poured upon the second filter, while the third filter is used for *c*, *d*, and *e*. After being well washed with the alcoholic ice-water, the whole of the asbestos filters and the sand in beaker *a* are repeatedly boiled with water, the extracts filtered, the united filtrates concentrated by evaporation, and the residue used for the determination of the gelatin nitrogen.

v. *Nitrogen in the Form of Flesh Bases and Decomposition Products Soluble in Alcohol.*—Five grammes of the dry preparations are warmed in a beaker with 25 c.c. of water. In the case of extracts 10 grammes are taken with 10 c.c. of water, and with fluid preparations from 20 to 25 c.c. are taken and no water used. Thin peptone solutions should be concentrated to about one-half their volume on the water-bath.

To the solutions 250 c.c. of absolute alcohol are gradually added with continual stirring. After standing from ten to twelve hours the liquid is filtered, and the residue repeatedly washed with alcohol. Leucine, tyrosine, and other decomposition products, together with part of the flesh bases, will be in solution. The alcohol is completely removed by distillation, the residue dissolved in water, and the solution filtered from the insoluble matter. The nitrogen of the insoluble residue is determined and added to the albumose nitrogen subsequently determined.

The filtrate is diluted to 500 c.c. and 100 c.c. taken for the estimation of the total nitrogen present, and a similar amount for the ammoniacal nitrogen. The difference between the two results gives the nitrogen present in the form of flesh bases and decomposition products.

vi. *Treatment of the Residue Insoluble in Alcohol.*—The filter containing the insoluble residue from v. is washed with water into a beaker, the alcohol evaporated on the water-bath and the liquid filtered. A small quantity of the albumoses usually becomes insoluble through the action of the alcohol, and the nitrogen in this must be determined and added to the albumose nitrogen subsequently found.

The filtrate is made up to 500 c.c., of which 50 c.c. are taken for the total nitrogen, 50 c.c. for the albumose, gelatin, and peptone, and 100 c.c. for the peptone alone.

The remainder of the liquid is concentrated by evaporation and tested for peptones by the biuret reaction applied to the filtrate obtained after precipitating the albumose and gelatin by saturating the liquid with ammonium sulphate.

vii. *Pancreas Peptone.*—The solution obtained in vi. contains, in addition to gelatin and albumose, the entire pancreas peptone. One hundred c.c. of the aqueous solution are evaporated to about 8 or 10 c.c., the gelatin and albumose precipitated by the addition of at least 100 c.c. of a cold saturated solution of ammonium sulphate, the precipitate washed with the same solution and dissolved in boiling water. The solution is then evaporated to dryness with barium carbonate to expel all the ammonia, the barium sulphate and carbonate removed, and the nitrogen found in the filtrate taken as that of pancreas peptone.

viii. *Albumose Peptone.*—A small quantity of this will have been found in v. and vi., but the bulk is present in the solution in vi. Fifty c.c. of this are mixed with an equal volume of dilute sulphuric acid (1 : 3), and phosphotungstic acid added in the cold so long as a precipitate forms. The precipitate is washed with dilute sulphuric acid and its nitrogen determined. This consists of nitrogen in the form of albumoses, pancreas peptones, and gelatin, of which the last two have already been determined. The difference gives the nitrogen in the form of albumoses, and to this must be added the small amounts found in v. and vi.

ix. *Nitrogen in the Form of Flesh Bases insoluble in Alcohol.*—This is obtained by taking the difference between the total nitrogen of vi. and that found in viii., after precipitation with phosphotungstic acid.

The analyses on p. 196 are given by Stutzer* to illustrate his method.

The chief objection brought against this method is the inaccuracy of the phosphotungstic acid precipitation (*cf.* page 167), since undoubtedly in many cases it causes a considerable proportion of meat bases to be classed among the peptones. Moreover, it is doubtful from the experiments of König and Bömer and of Allen whether peptones (or, in other words, hydrolysed proteids precipitated by bromine but not by saturation with ammonium or zinc sulphate) are ever present in more than traces in meat extracts properly so called.

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* *Zeit. angew. Chem.*, 1895, p. 157.

	Liebig's Extract.	Kemmerich's Extract.	Bovril Fluid Beef.	Bovril Fluid Beef, Seasoned.	Bovril for Invalids.	Bovril Beef Jelly.	Bovril Lozenges.
Water,	17·72	16·54	29·14	44·42	28·13	89·15	9·47
Sodium chloride,	3·11	4·15	14·12	10·72	4·57	0·26	1·63
Other salts,	19·63	17·96	3·38	7·60	11·50	1·04	5·71
Organic matter,	59·64	61·35	53·36	37·26	55·80	9·55	83·19
Nitrogen was present as—							
a. Albumose peptone,	0·56	1·24	1·23	0·34	1·26	0·16	2·06
b. Pancreas peptone,	2·72	2·38	3·36	1·39	3·36	0·48	6·06
	3·28	3·62	4·59	1·73	4·62	0·64	8·12
c. Flesh bases, etc. soluble in alcohol,	4·05	3·69	1·06	1·16	1·78	0·21	0·55
d. Do. insoluble in alcohol,	1·34	1·25	1·16	0·89	0·82	0·20	1·16
	5·39	4·94	2·22	2·05	2·60	0·41	1·71
e. Albumin,	0·12	0·09	0·31	0·08	0·24	...	0·42
f. Muscular fibre,	...	...	0·73	0·90	0·70	...	0·57
	0·12	0·09	1·04	0·98	0·94	...	0·99
g. Gelatin,	0·04	0·05	0·09	0·09	0·15	0·29	0·70
h. Ammonium salts,	0·48	0·46	0·31	0·27	0·38	0·12	0·42
	0·52	0·51	0·40	0·36	0·53	0·41	1·12
Total nitrogen,	9·31	9·16	8·25	5·12	8·69	1·46	11·94

Although Schjerning asserts that he has found true peptones in considerable quantity in Liebig's Extract by his method of precipitation with various metallic salts, he does not prove the identity of the substances which he has separated with those which give the biuret reaction and are precipitated by bromine after the removal of albumoses from the solution (*cf.* pp. 159 and 204).

The recent analyses by Hehner given on p. 197 show the composition of a number of well-known preparations. They were made by a method essentially the same as that of Stutzer. Each

No.	Description.	Water.	Fat 'etroleum) rit Extract.	Gelatin.	lbumin.	eat Fibre Coagulable lbumin.	lbumoses.	eptones.	eat Bases.	Ash.	ifference.	Sodium hloride.	hosphoric Acid.	Total Nitrogen.
1	Liebig Company's *Extractum Carnis*, .	15·26	0·34	5·18	...	2·12	2·01	8·06	39·32	23·51	4·20	5·81	6·97	9·07
2	Armour's Extract of Meat, . . .	15·97	0·21	8·31	...	...	1·75	5·13	41·12	29·36	3·15	9·74	6·76	8·21
3	Brand & Co.'s *Extractum Carnis*, . .	17·85	0·38	4·56	...	1·81	4·19	10·16	38·90	18·80	2·87	3·31	5·16	9·80
4	Liebig's Extract (Bovril Co.'s make), .	22·24	0·29	5·50	...	1·30	3·62	8·44	38·59	20·45	−0·42	5·14	5·50	9·19
5	Brand & Co.'s Meat Juice, . .	55·48	0·10	0·69	1·00	...	1·06	2·50	12·50	11·06	15·61 Glycerin?	4·43	1·52	2·81
6	Valentine's Meat Juice, . . .	55·53	0·10	0·75	0·25	...	2·00	2·87	12·48	12·01	14·01	2·35	2·85	2·92
7	Wyeth's Meat Juice,	61·61	0·08	1·12	5·62	...	1·08	1·86	9·44	14·78	4·41	6·96	3·01	3·06
8	Borthwick's Bouillon,	36·19	0·25	1·37	...	4·00	1·16	11·09	24·25	17·93	3·76	6·09	3·58	6·70
9	Vitalia Meat Juice, .	70·19	0·32	0·45	16·44	0·37	0·05	0·37	2·82	6·65	2·34	5·11	0·37	3·28
10	Brand & Co.'s Essence of Beef, . .	89·69	0·06	5·18	...	...	0·19	0·57	3·43	1·00	−0·05	0·33	0·40	1·49
11	Bovril Co.'s Fluid Beef, . . .	28·34	1·02	3·81	...	5·37	8·38	13·18	19·38	17·67	2·85	9·07	4·05	8·02
12	Bovril Co.'s Fluid Beef (unseasoned),	44·75	0·62	1·06	...	7·31	2·38	6·25	17·12	19·90	0·61	11·42	3·34	5·46
13	Bovril for invalids, .	24·34	1·07	4·56	...	5·87	5·56	6·44	34·07	16·50	1·59	5·23	3·35	9·20
14	Bovril for invalids, .	17·47	0·51	2·56	4·43	15·25	1·06	8·82	31·89	16·30	1·91	2·46	1·43	10·21
15	Caffyn's *Liquor Carnis*, . .	48·46	0·11	0·25	2·19	0·94	3·65	0·98	11·30	9·95	22·17 Glycerin	4·43	0·62	3·09
16	Extract of Meat with vegetable extracts, .	30·03	0·10	1·69	6·12	...	1·74	4·85	16·97	23·47	15·05 Carbo-hydrate	11·56	3·02	5·02

of the nitrogenous constituents was calculated from the nitrogen as determined by Kjeldahl's method, the factor 6·25 being used in every instance:—

König and Bömer obtained the results given in the subjoined table in the course of their critical examination of Stutzer's and Kemmerich's methods (pp. 193 and 201).

They assigned the nitrogen found as follows:—

	Liebig's Extract.		Kemmerich's Extract.		Kemmerich's Peptone.		Cibil's Extract.	
Total Nitrogen Found, .	9·28		9·14		10·08		2·77	
	Percentage on Total Substance.	Percentage Proportion of Total N.	Percentage on Total Substance.	Percentage Proportion of Total N.	Percentage on Total Substance.	Percentage Proportion of Total N.	Percentage on Total Substance.	Percentage Proportion of Total N.
Distributed as follows:—								
1. Soluble albumin, . .	trace	trace	0·08	0·87	0·06	0·59	trace	trace
2. Nitrogenous constituents insoluble in 60 to 64 per cent. alcohol, .	0·21	2·26	0·33	3·61	1·36	13·49	0·25	9·02
3. Albumoses, . . .	0·96	10·34	1·21	13·24	4·15	41·17	0·70	25·27
4. Peptones, . . .	0 to trace	0 to trace	0	0	0	0	0	0
5. Flesh bases, . . .	6·81	73·38	5·97	65·32	3·97	39·38	1·56	56·31
6. Ammonia, . . .	0·47	5·06	0·41	4·49	0·29	2·88	0·09	3·25
7. Other nitrogenous compounds, . . .	0·83	8·96	1·14	12·47	0·25	2·49	0·17	6·15

As regards the chemical examination of meat extracts in general they remark—

1. Precipitation with 80 per cent. alcohol does not differentiate the kinds of nitrogen.
2. Albumoses should be determined by saturating their solution with ammonium sulphate or zinc sulphate.
3. The filtrate from the albumoses should be decolorised with animal charcoal and tested for peptones by the biuret reaction.
4. Ammonia may be estimated by distilling the solution with ignited magnesia.
5. When peptone has been proved to be absent, the nitrogen in the phosphotungstate precipitate, after deducting the nitrogen derived from gelatin, albumoses, and ammonia, may be ascribed to the flesh bases. The precipitate should stand at least one day.
6. The difference between the total nitrogen and the nitrogen in the form of gelatin + albumoses + flesh bases + ammonia gives the amount of nitrogen contained in the compounds not precipitated by phosphotungstic acid.

Application of Formaldehyde to the Analysis of Commercial Peptones, etc.—E. Beckmann has examined a large number of peptones, commercial 'peptones,' and meat extracts by his method described on p. 175, and finds that as a general rule the amount of insolube residue in meat extracts does not exceed 3 per cent.

Preparation.	Insoluble Formalin Residue. Per Cent.	Containing Proteid. Per Cent.
Peptone e carne (Merck), . . .	0·51	0·29
Peptone puriss. (Grübler), . . .	trace	trace
Peptone from Albumin (Merck), .	11·07	3·60
Peptone sicc. from blood fibrin (Merck),	20·50	4·36
Hydropeptone (Merck), . . .	1·45	0·76
Kemmerich's Flesh Peptone, . .	14·15	1·92
Denaeyer's Peptone, ...	13·87	0·48
Bovril Lozenges peptonised, . .	46·49	5·96
Liebig's Meat Extract, . . .	0·47	0·25
'Santa Maria' Extract (Liebig), .	1·03	0·55
Kemmerich's Extract, . . .	0·84	0·26
Maggi's Meat Extract, . . .	2·33	0·80
Cibil's Extract (solid), . . .	1·10	0·20
,, ,, (liquid), . . .	1·05	0·47
Armour's Meat Extract, . . .	2·09	1·29
Bovril Fluid Beef, seasoned, . .	3·25	...

Analyses by Alcohol Precipitation.—The fact that different proteids dissolve in different strengths of alcohol has been made the bases of several methods of examining meat extracts. In the earliest of these, all that was attempted was to effect an incomplete separation of proteids and gelatinous substances from the meat bases, which are rightly considered to be the most important constituents in meat extracts.

O. Hehner* analysed a number of preparations by this method in 1885. Two grammes of the sample were dissolved in 25 c.c. of water, and 50 c.c. of strong methylated spirit added to the solution. After standing overnight, the clear supernatant liquid was decanted from the precipitate, the latter dissolved

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* *Analyst*, x. p. 221.

(without washing) in hot water, the solution evaporated in a weighed basin, and the residue dried at 100° C. and weighed.

Some of the results thus obtained were :—

Preparation.	Water.	Total Solids.	Alcohol Precipitate.	Ash.	Phosphoric Acid.	Nitrogen.
Liebig's Extract, . .	18·70	81·30	5·16	23·38	6·07	7·94
Nelson's Gelatin, . .	...	...	93·19	3·25	none	...
Concentrated Beef Tea.						
English, ...	36·96	63·04	27·40	4·36	1·16	8·25
English, ...	31·00	69·00	30·30	4·13	1·00	8·36
English, ...	41·93	58·07	25·50	4·92	1·10	7·52
Russian, ...	24·56	75·44	35·40	6·72	0·95	9·89
X, ...	54·31	45·69	32·30	7·57	2·11	6·79
Commercial Essence of Beef.						
English, ...	89·25	10·75	3·07	1·17	0·34	1·36
English, ...	89·61	10·39	3·74	1·00	0·26	1·36
English, ...	92·32	7·68	1·99	1·30	0·38	0·79

The general conclusions arrived at by Hehner from a consideration of these results were that the amount of ash should be considerable, and should contain 25 per cent. of phosphoric acid, and that the substances precipitated by alcohol should not exceed 25 per cent. of the total dry solids of meat essence, or 44 per cent. of those of beef tea.

Kemmerich[*] made use of fractional precipitation with alcohol of different strengths in order to effect a separation of the nitrogenous constituents in meat extract, and by this means found the following quantity of proteids, in addition to flesh bases, in South American meat extract.

1. Gelatin, precipitated by 50 to 60 per cent. alcohol,	6·19
2. Albumoses, precipitated by 80 per cent. alcohol, .	14·76
3. Peptones, soluble in 80 per cent. alcohol. Precipitated by sodium phosphotungstate, . .	12·31
	33·26

König and Bömer critically examined Kemmerich's work, but instead of weighing the precipitates as he had done, determined the nitrogen they contained and calculated the proximate constituents from the result. In this way they obtained considerably lower results than Kemmerich.

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[*] *Zeit. physiol. Chem.*, 1894, xviii. p. 409.

	Gelatin (?) precipitated by 50 to 60 per cent. alcohol.		Albumoses precipitated by 80 per cent. alcohol.
Kemmerich, . .	6·19	...	14·16
König and Bömer, .	1·83	...	4·50

They found that the filtrate left after precipitation with 80 per cent. alcohol gave the biuret reaction, showing that proteids were still present, but considered it questionable whether these were peptones, for on saturating the solution with ammonium sulphate and testing the filtrate there was no biuret reaction, which should have been the case with peptones.

The following comparative results given by the precipitation with 80 per cent. alcohol, and by 'salting out' with ammonium sulphate, showed that albumoses were incompletely precipitated by the former process.

	Liebig's Extract. Per Cent.	Kemmerich's Extract. Per Cent.	Kemmerich's Peptone. Per Cent.	Cibil's Extract. Per Cent.
Total Nitrogen, . .	9·32	8·94	9·88	2·77
Nitrogen precipitated by 80 per cent. Alcohol, .	0·69	1·05	4·05	0·61
Corresponding to Albumoses, . . .	4·31	6·56	25·31	3·81
Albumoses salted out with Ammonium Sulphate, . . .	7·32	9·71	34·44	5·97

By precipitating the nitrogenous constituents with sodium phosphotungstate, and deducting the albumose nitrogen determined in the ammonium sulphate precipitate, a large residue was obtained, which has often been regarded as consisting exclusively of peptones.

	Liebig's Extract. Per Cent.	Kemmerich's Extract. Per Cent.	Kemmerich's Peptone. Per Cent.	Cibil's Extract. Per Cent.
Nitrogen in Phosphotungstate Precipitate,	6·27	5·59	8·29	2·00
Albumose Nitrogen, .	1·17	1·55	5·51	0·96
Peptone (?) Nitrogen, .	5·10	4·04	2·78	1·04

From the method of preparation it was obvious, in the case of meat extracts at least, that so large an amount of peptones could not have been present, and that the meat bases, which, as is well known, are also precipitated by sodium phosphotungstate, must have accounted for a considerable proportion, if not all of the nitrogen assigned to the peptones.

From these considerations König and Bömer arrived at the conclusion that precipitation with 80 per cent. alcohol is of no value in determining the kind of nitrogen.

They also expressed doubt as to the correctness of the amount of gelatin (precipitated by 50 to 60 per cent. alcohol) found by Kemmerich. They argued that since meat extracts are prepared at low temperature and only concentrated after filtration, the quantity of gelatin could only be excessively small, and in support of this view referred to the experiments* of E. Beckmann, who could only find 0·5 per cent. of albumin and gelatin in Liebig's Extract by precipitation with formaldehyde.

A similar method of examining meat extracts has been described by J. Bruylant,† who precipitates the gelatin by 40 per cent. alcohol, albumoses by 80 per cent. alcohol, and peptones by alcohol of from 93 to 94 per cent.

The results thus obtained with certain preparations were:—

	Liebig's Extract.	Solid Bovril.	Bovril for Invalids.	Fluid Bovril.
Water, ...	16·75	19·20	2·35	43·25
Sodium Chloride, ...	2·95	4·50	4·00	9·75
Other Salts, ...	18·24	16·20	17·05	6·25
Insoluble in Water (Meat Fibre), .	..	..	7·10	8·19
Organic Matter, ...	62·06	60·10	54·50	82·06
Total Nitrogen, ...	9·30	8·85	9·12	4·85
Nitrogen in portion insoluble in water, ...	..	..	1·09	1·19
Nitrogen (Ammoniacal, from Uric Acid, &c.), ...	0·60	0·50	0·45	0·30
Nitrogen, from Lead Precipitate (Non-Proteid Substances), . .	0·65 } 5·09	0·57 } 4·56	0·45 } 4·48	0·27 } 2·67
Nitrogen, Non-Proteid, from 80 per cent. Alcohol, ...	0·15	0·20	0·18	0·05
Nitrogen, soluble in strong Alcohol,	3·60	3·29	3·40	1·05
Nitrogen from Gelatin, . . .	0·19	0·25	0·12	0·05
,, ,, Albumoses, . .	0·80 } 3·93	0·95 } 3·78	0·75 } 3·57	0·45 } 1·83
,, ,, Peptones, . .	2·94	2·58	2·70	1·33
Total Soluble Proteids, . . .	24·56	23·62	22·40	11·43
Insoluble Albumin (Meat Fibre), .	..	..	6·81	7·43

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[*] *Forschungs Ber.*, 1894, p. 423; *cf.* page 199.

[†] *Journ. Pharm. Chim.*, 1897, v. p. 515.

KÖNIG'S ANALYSES OF FLESH 'PEPTONES.'

	Water.	Organic Matter.	Total Nitrogen.	In Organic Matter.					Salts.	In the Salts.			In 80 per cent. Alcohol.	
				Insoluble Proteids N×6·25.	Propeptones.	Peptones N×6·25.	Other Nitrogenous Compounds.	Fat = Ether Extract.		Potassium.	Phosphoric Acid.	Chlorine or NaCl.	Soluble.	Insoluble.
E. Merck's (pancreas) peptone— *a.* Syrup, . .	32·42	63·75	9·01	trace	10·75	27·94	24·67	0·39	3·83	1·78	1·46	...	52·40	15·18
b. Powder, .	6·91	86·76	13·26	0·63	23·00	32·49	30·03	0·61	6·33	...	...	...	82·87	10·12
Cibil's (papayotin) flesh peptone, .	26·77	58·27	9·51	0·27	5·27	39·45	18·20	0·35	14·97	4·10	3·23	Cl 4·55	...	...
Cibil's flesh solution,	23·75	49·22	8·45	0·43	3·52	34·76	10·94	...	26·98	7·93	6·11	5·34	...	...
Antweiler's (papayotin) peptone, . .	6·92	89·78	12·85	3·22	14·54	60·15	1·20	0·54	13·31	0·68	0·50	9·63	...	...
Kemmerich's peptone (steam), . .	33·30	58·47	9·78	1·10	14·56	32·57	9·97	0·30	7·73	3·32	2·49	0·66	26·32	40·88
Koch's peptone (steam), . .	61·87	21·71	3·50	0·38	7·16	6·09	7·03	1·05	16·42	2 35	1·69	7·62	...	...

The criticisms of König and Bömer on Kemmerich's work are in the main also applicable to Bruylant's method. At best the separation is not sharp, and variations in the strength of alcohol would alter the proportion of the constituents in each group. In some cases in which the decomposition of the albumin molecule had been carried slightly further, products of lower molecular weight and of greater solubility would be grouped with the peptones, although by saturating their solution with zinc sulphate or ammonium sulphate they would be found among the albumoses.

Although by alcoholic precipitation concordant results can be readily obtained, which will indicate more or less accurately the degree of hydrolysis which the original proteid molecule has undergone, the method is much less convenient and exact than separations effected by means of zinc sulphate and bromine.

Older Analyses of Flesh Peptones.—König* gives the foregoing analyses (see p. 203) of preparations of this class. As the propeptones (albumoses) were estimated by precipitation with ferric acetate, and the peptones by precipitation with phosphotungstic acid, the nitrogenous constituents returned as peptones probably contained a large proportion of albumoses as well as of meat bases.

By precipitating the albumoses by saturation with ammonium sulphate König obtained the following amount of albumoses and peptones from Cibil's and Antweiler's peptones.

	Albumoses.	Peptones.
Cibil's *a*, ...	13·71	28·29
,, *b*, ...	6·51	9·62
Antweiler's	47·74	27·10

Schjerning's Method.—The precipitation of the proteid substances in meat extracts, etc., in the form of distinct metallic compounds, is carried out as described on page 171.

In a recent communication to the author, Schjerning states that he includes the peptones under the term of 'protein substances,' and that he defines them as 'proteids not precipitated from a neutral or acetic acid solution on moderately warming the liquid after saturation with a readily soluble sulphate.' For the saturation he prefers magnesium sulphate, finding that it precipitates the same quantity of proteid nitrogen as zinc or ammonium sulphates. Schjerning's peptone therefore agrees with Kühne's definition of a true peptone (but *cf.* page 196).

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* *Nahr. u. Genussm.*, II. p. 186.

'Denuclëin,' he asserts, is not a proto-albumose, since all albumoses are precipitated from an acetic acid solution by saturation at 30° to 36° C. with a readily soluble sulphate, whilst denuclëin is not thus precipitated. It is rather, as its name denotes, a lower nuclëin compound, and possibly a nucleïc acid substance.

In support of Schjerning's position it may be pointed out that in the table of König and Bömer's results (page 198), about 9 per cent. of the total nitrogen in Liebig's Extract is not classified under the head of albumin, albumoses, peptones, flesh bases, or ammonia, but belongs to other nitrogenous compounds.

Some of Schjerning's results are shown in the subjoined tables:—

I.

Precipitation with	Liebig's Flesh Peptone.		Witte's Peptone.		Liebig's Extract. 1896.	Liebig's* Extract. 1899.
a. $SnCl_2$.	13·0	13·5	8·0	3·0	10·7	5·5
$Pb\bar{A}c_2$.	†	†	†		19·2	...
b. $HgCl_2$.	24·8	24·5	84·0	84·0	...	14·8
c. $Fe\bar{A}c_2$.	57·2	56·6	59·4	...	25·3	24·7
d. $U\bar{A}c_2$.	55·3	55·9	55·9	55·1	36·7	32·3
e. $MgSo_4$.	48·1	48·1	47·2	47·2	15·1	15·1
Allowable Error Per Cent.	0·5		0·8		0·8	0·5

II.

	Liebig's Flesh Peptone.		Witte's Peptone.		Liebig's Extract. 1896.	Liebig's Extract. 1899.
Albumin I.,	13·0	13·5	3·0	3·0	10·7	5·5
Albumin II.,	3·4	2·5	18·8	} 31·0	−1·7	−0·3
Denuclëin, .	8·4	8·5	12·2		10·2	9·6
Albumoses, .	32·4	32·1	25·4	} 21·1	6·1 } 17·5	9·9 } 17·5
Peptones, .	−1·9	−1·6	−3·5		11·4	7·6

It is remarkable that the combined amount of the nitrogen from the albumoses and 'peptones' should be identical in the two samples.

* Unpublished. † The precipitation could not be made.

The residual nitrogen representing the flesh bases, amido-compounds, ammonium salts, etc., was calculated to be 63·3 per cent. in 1896 and 67·7 per cent. in 1899. It is suggestive that König and Bömer assigned about 10 per cent. more nitrogen to the flesh-bases and found no peptone nitrogen, whilst Schjerning obtained about 10 per cent. of the latter (p. 198).

Although in certain cases Schjerning's process may eventually be found a satisfactory method of fractionating the proteid nitrogen in different organic substances, it suffers at present from the drawback of being new and of yielding results which are difficult to compare with those of older methods.

Probably the metallic compounds obtained by it are of a more definite character than those which, like the compounds yielded by saturating the solution with salts, are only separated from one another by a difference in solubility.

If this be the case, and if the physiological characteristics of the various proteids precipitated in combination with the metals be determined, a distinct advance will have been made in estimating the comparative value of different meat extracts and similar preparations.

CHAPTER X.

THE COOKING OF FLESH.

Advantages of Cooking.—The process of cooking has three main advantages:—(1) It renders the flesh more appetising by the development of certain odours and flavours under the action of the heat; (2) it destroys animal parasites, and to a certain extent bacteria and bacterial products; and (3) the flesh is eaten at a temperature more favourable to the action of the gastric juice. On the other hand, the digestibility of cooked meat is considerably less than that of raw meat, as was shown by Chittenden and Cummins,[*] who found that if the digestibility of cooked beef in artificial pepsin solution be taken as 100, that of raw beef may be represented by 142·38. The longer the cooking has been continued, the greater is the decrease in digestibility.

The national economy of invariably eating flesh in a cooked condition is shown by the fact that Berlin employs over 200 trichinæ inspectors, and Prussia more than 24,000, as a safeguard against one of the dangers of raw flesh,[†] whereas in other countries, such as Italy, France, and England, where pork is, as a rule, only eaten in the cooked state, no special inspectors are employed, and yet trichinosis is of comparatively rare occurrence. In Germany the public is now warned against eating raw meat, even after it has been inspected and passed.

The Loss during Cooking.—This depends to a large extent on the method of cooking. According to Letheby,[‡] the average percentage loss in weight on boiling is 23; in baking, 31; and in roasting, 34. If the meat is placed in cold water which is gradually heated to the boiling-point, the loss is considerably higher, owing to the large proportion of soluble proteids, nitrogenous extractives, and mineral matter, which dissolves before the temperature (50° to 70° C.) is reached, at which the albumin begins to coagulate and to form a protective crust on the surface. Under these circumstances the loss may amount to from 30 to 40 per cent. (Strohmer). Part of the loss during boiling is due to some of the collagene being converted into gelatine and dissolving in the water.

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[*] *Journ. Amer. Chem. Soc.*, vi. p. 318; *cf.* page 86.

[†] *Der Fleischbeschau*, p. 547.

[‡] *On Food*, p. 166.

In roasting and baking the constituents of the flesh are retained much more completely than in boiling, and although the loss is apparently greater, it is almost entirely due to water. The figures given by Strohmer* as representative of the average loss in different kinds of flesh on roasting are considerably lower than those of Letheby—viz., Beef, 19 per cent.; veal, 22 per cent.; mutton and poultry, 24 per cent.

The Composition of Cooked Meat.—*Roasting.*—When meat is roasted there is a considerable loss in weight, amounting from 25 to 35 per cent. calculated on the original substance. This is mainly due to the evaporation of water and to loss of the substance of the meat in the form of dripping and gravy.

By maintaining a high temperature during the initial stages of the process, the juice which first escapes from the meat becomes coagulated on the surface and furnishes a thin glaze, which prevents any further considerable loss of meat juice.

Some idea of the difference in composition of raw and roasted meat may be obtained from the following analyses. As they represent cuts from different joints, they are not, however, strictly comparable:—

	Water.	Nitrogenous matters.	Fat.	Ash.
Beef, raw, ...	71·68	20·52	6·72	1·21
,, roasted, ...	50·82	25·05	21·65	1·45
Mutton, raw (König), . . .	75·99	17·11	5·77	1·33
,, roasted (Mitchell), .	45·21	31·84	21·37	1·58

The characteristic aromas of roast meat are due to the partial carbonisation of the meat fibre with the formation of odorous compounds.

Boiling.—The change which meat undergoes during boiling differs very much from that which takes place in roasting. The loss in water is much less, but the action of the boiling water on the collagene of the connective tissue causes a large amount of gelatin to be dissolved. The meat also loses much of its extractives and mineral matter, which will be found in the broth. This loss can be obviated to some extent by first placing the meat in boiling water, so as to coagulate the myosin of the muscular tissue, and thus prevent the loss of much of these constituents during the subsequent gentle boiling or simmering. When gravy or soup are required the opposite process is followed, the meat being placed in water of a low temperature, which is gradually heated, though not to the boiling-point.

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* *Die Ernahrung des Menschen*, p. 123.

According to Pereira,* the average loss in weight in the joints of beef and mutton boiled for the inmates of the Wapping warehouse was 17·5 per cent., but in Letheby's opinion this was considerably lower than the general average.

O. W. Andrews states that the total loss on cooking should not exceed one-fifth to one-fourth for boiled meat and about one-third for roast meat.

Grilling and Frying.—Grilled meat resembles roast meat in many respects, but owing to the more thorough and direct action of the heat, there is a greater loss of water and of dripping, and a more complete carbonisation of the exterior meat fibre.

In frying, which may be regarded as boiling in fat, the presence of the latter modifies the direct action of the fire.

A. H. Church † gives the following analyses of raw and of cooked mutton chops:—

Fresh mutton chop, minus bone—Water, 44·1; albumin, 1·7; fibrin (true muscle), 5·9; ossein-like substances, 1·2; fat, 42·0; organic extractives, 1·8; mineral matter, 1·0; and other substances, 2·3 per cent.

COMPOSITION OF TWO COOKED MUTTON CHOPS.

	Water.	Nitrogenous Matters.	Fat.	Mineral Matter.	Other Substances.
I. Chop, including gravy and dripping, . . .	54·0	27·6	15·4	3·0	...
II. Chop, without gravy and dripping, . . .	51·6	36·6	9·4	1·2	1·2

"Meat is tender if properly cooked *before* the *rigor mortis* has set in, but must be kept for some days *after* that rigidity of the muscles has set in," if the same degree of tenderness be required.‡

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* Letheby, *On Food.*

† *Food: Some Account of its Sources*, p. 166.

‡ Church, *loc. cit.*, p. 163.

The Composition of Cooked Fish.—In a recent communication to the Chemical Society * Miss K. Williams gave the following results (among others) of her analyses of different kinds of boiled fish as they would be served at table. The salt cod and herrings were soaked in cold water before cooking, and the sardines well washed in boiling and cold water to remove as much surface oil as possible. When cold the inedible portions (bones, head, skin, etc.) were removed, weighed, crushed in a mortar, boiled in distilled water, the liquid siphoned off and evaporated on a water-bath. The residue was dried until constant in weight and taken as gelatin.

Name of Fish.	Date.	Portion Analysed.	As served at Table.			
			Waste—Bones, etc.	Gelatin.	Water.	Nutrients.
Herrings, .	Feb.	Whole	11·74	0·63	52·99	34·54
Salt herrings,	Jan.	Flesh	...	...	46·03	53·97
Sardines, .	March	Whole	4·91	...	42·17	52·92
Sprats, . .	Nov.	,,	17·90	0·90	61·50	19·70
Salmon, .	July	Section	5·99	0·53	61·06	32·02
Eels, . .	Oct.	Heads removed	11·66	1·09	53·29	33·96
Mackerel, .	April	Whole	10·51	0·25	65·21	24·03
Cod, . .	Jan.	Section	15·99	0·43	63·78	19·79
Salt cod, .	Feb.	,,	6·13	0·33	67·68	25·86
Haddock, .	Jan.	Whole	35·10	0·80	46·46	17·64
Turbot, . .	Feb.	Anterior and head	31·20	0·59	53·09	15·12
Plaice, . .	Dec.	Flesh	...	...	79·86	20·14
Soles, . .	March	Whole	22·02	0·74	61·18	16·06
Oysters, .	,,	Shell contents	...	...	77·71	22·29

A further analysis of the same specimens of fish gave the additional results given on page 211.

The amount of reducing substances was obtained by removing the fat with benzene, and digesting the residue with 100 c.c. of water and 10 c.c. of hydrochloric acid (sp. gr. 1·125) on the boiling water-bath under a reflux condenser for three hours. The liquid was then filtered, basic lead acetate added, and a current of sulphur dioxide passed through the filtrate. The solution was again filtered, concentrated, and washed alumina added until it no longer dissolved. After filtration the liquid was evaporated to dryness at 100° C., the residue treated with boiling alcohol, the liquid filtered, and the alcohol removed by evaporation. The residue was dissolved in water, the liquid boiled with animal charcoal and a few drops milk of lime, filtered, and titrated with Fehling's solution.

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* *Journ. Chem. Soc.*, 1897, p. 652.

With reference to the results thus obtained, H. A. Allen points out that the reducing substances were probably not present as such, but were products of the hydrolysis of gluco-proteids by the hydrochloric acid.

Name of Fish.	Water in Flesh.	Analysis of the Dried Substances.					
		Ash.	Fat (ether extract).	Proteids (N × 6·25).	Reducing Substances as Glucose.	Phosphorus.	Nitrogen pentoxide.
Herrings,	60·54	5·56	25·25	67 07	...	0 91	0·66
Salt herrings, .	46·03	19·69	21·90	38·88	17·59	0·89	1·64
Sardines,	44·35	12·03	33·49	55·44	...	0·97	...
Sprats, .	75·77	6·42	27·37	57·94	9·88	1·17	...
Salmon, .	65·32	4·94	29·43	56·65	14·89	0·51	0·46
Eels, .	61·08	2·11	44·68	42·88	8·91	0·42	...
Mackerel,	73·13	4·07	25·73	62·32	13·93	0·85	0·33
Cod, .	76·32	3·31	1·15	91·55	6·67	0·62	0·63
Salt cod,	72·35	14·26	0·94	76·06	7·14	0 29	0·31
Haddock,	72·37	8·28	1·29	79·57	13·15	0·53	0·43
Turbot, .	77·84	2·41	4·75	84·71	11·81	0·57	...
Plaice, .	76·86	4·06	9·84	75 16	11·56	0·71	2·78
Soles, .	79·20	3·47	1·71	86·71	11·87	0·52	...
Oysters,	77·71	12·16	7·77	65·42	18·32	0·49	...

He also calls attention to the fact that these analyses do not confirm the popular belief that the amount of phosphorus in fish is very much greater than that of meat.

The Effect of Cooking on Animal Parasites.—The experiments of Perroncito (pages 248 and 261) and others have shown that the cysticerci and other larvæ of the tapeworms perish below 50° C., trichinæ below 69° C., and that no animal parasite found in flesh is capable of withstanding as high a temperature as 70° C. If, then, this temperature is reached in every part of the meat during the cooking, all risk from this source is obviated.

The Temperatures at which Bacteria Perish.—Bacteria are much more resistant to the action of heat, especially of dry heat, than are the animal parasites found in flesh. Generally speaking, the pathogenic bacteria perish at a lower temperature than the non-pathogenic bacteria. Sternberg[*] exposed pure cultivations of various micro-organisms for ten minutes at different temperatures and obtained the following thermal death points:—

	°C.		°C.
Bacillus of Swine Erysipelas,	58	Staphylococcus pyogenes albus,	62
Bacillus pyocyaneus,	56	Staphylococcus pyogenes citreus,	62
Bacillus prodigiosus,	58	Streptococcus pyogenes aureus,	54
Bacillus fluorescens,	54		
Bacillus acidi lactici,	56		
Staphylococcus pyogenes aureus,	58		

The following results have been obtained by other observers at different times:—

B. of Swine Erysipelas.—Killed in 5 minutes at 55° C. in pure cultivations, but not destroyed in meat by ordinary cooking (Petri).

B. of Hog Cholera.—15 minutes at 70° C. One hour at 54° C. (Smith).

B. of Rabbit Septicæmia.—15 minutes at 55° C.; 10 minutes at 80° C. (Ostertag).

B. anthracis.—10 minutes at 54° C., or 20 minutes at 50° C. (Chauveau). 10 to 15 minutes at 55° to 60° C. (Besson). The spores failed to grow after 4 minutes at 100° C. (Sternberg). Spores destroyed by moist heat at 90° to 95° C., but capable of withstanding a much higher dry temperature (Besson).

B. of Quarter Evil.—Virulent after an hour at 80° C. Killed after 5 minutes at 100° C. The spores only weakened in a current of steam (Ostertag).

B. of Glanders.—Killed at 55° C. (Löffler); 55° to 60° C. (Besson).

B. tuberculosis.—Perishes at 85° in pure cultivations (Bang). Can withstand 65° C., but perishes at 75° C. (Yersin). In milk, 4 hours at 55° C.; 1 hour at 60° C.; 15 minutes at 65° C.; 5 minutes at 80° C.; 1 minute at 95° C. (Forster). In the dry state resists 100° C. for 3 hours, and 70° C. for 7 hours (Welch).

B. tetani.—Killed after 6 hours at 80° C.; 2 hours at 90° C.; and 8 minutes at 100° C. (Besson). The spores are extremely resistant to heat.

Streptococcus pyogenes aureus.—One hour at 58° C., and a few moments at 100° C. (Besson).

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[*] *Bacteriology*, p. 147.

Staphylococcus pyogenes aureus. " " *albus.* " " *citreus.*	Dead after 24 hours at 55° C., or 15 minutes at 80° C. (Besson).

Bacillus coli communis.—5 minutes at 66° C. (Besson).

Bacillus typhosus.—From 10 to 20 minutes at 66° C. in pure cultivations (Besson).

Action of Heat on Bacterial Toxines.—The excretory products of pathogenic bacteria consist as a rule of several active principles. They sometimes contain ptomaines apparently identical with those formed by purely putrefactive bacteria, sometimes definite bases only known to be formed by specific bacteria, together with various broken-down products of albuminous substances (toxalbumoses, peptones, etc.). In some cases definite toxic products have been isolated from pure cultivations (*cf.* page 220), but as a rule the experiments as to the influence of heat have been made with the bouillon filtered free from bacteria and containing mixed toxic and harmless products.

The following are some of the results which have been obtained:—

Toxic Products of Staphylococcus pyogenes aureus.—The activity of the whole toxine is weakened at 58° C. There are two active principles in the cultivations, one precipitated by alcohol and destroyed at 104° C., the other not precipitated by alcohol and unweakened at 104° C. (Besson).

Toxines of Tuberculosis and Glanders.—One or more of the active toxic principles in the products of *B. tuberculosis* and *B. mallei* are not destroyed at 100° C., as is shown by the method of preparing crude tuberculin and crude mallein.

Toxine of Rinderpest.—Destroyed after 10 minutes at 55° C. (Semmer and Raupach).

Toxine of Sheep pox.—10 minutes at 55° C. (Semmer and Raupach).

Virus of Rabies.—10 minutes at 60° C. (Sternberg).

Toxine of Anthrax.—Weakened but not destroyed at 100° C.

Toxine of Tetanus.—Altered by heating at 65° C. for 5 minutes, and toxicity completely destroyed at 80° C. (Kitasato).

The products of several of the bacteria of septicæmia have been shown to be toxic after boiling, and the same remark applies to many of the putrefactive poisons. Hence cooking, even if a temperature of 100° C. were reached in every part of the meat, cannot be regarded as a universal safeguard against bacterial poisons.

Lehmann regards flesh infected with the following diseases as dangerous only in the raw or unperfectly cooked condition:—Cysticerci, trichinæ, tuberculosis, glanders, actinomycosis and foot-

and-mouth disease. He considers flesh infected with splenic fever, malignant œdema, septicæmia, and chicken cholera as dangerous whether raw or cooked.

The Temperatures reached in the Ordinary Process of Cooking.—Some of the experiments which have been made with the object of determining this point are given on page 262, where it is shown that cooking, if thoroughly carried out, destroys trichinæ.

In further illustration of the fact that heat penetrates but slowly into the interior of flesh, the experiments of other observers* may be described. Rupprecht found that in the ordinary boiling of meat for ¾ hour, as in Saxony, the interior temperature was at most 75° C. at the end of the time, and that, too, only when the meat was in thin strips. In blood sausage the temperature reached in the same time was 66° C.; in tongue sausage 62·5°; in ham 65°; and in boiled pork 65°. The interior temperature of a rapidly-roasted sausage was only 28·7° C.

Leuckart found that in grilled cutlets and sausages the highest temperature was 62·5° C., and that of roast pork 75° C.

Wolffhügel and Hueppe state that the temperature in the middle of large pieces of meat never reaches 100° C., and in their experiments this temperature was only once attained in the exterior parts.

From these experiments it follows that many of the bacteria, if present in the interior of flesh, would probably survive the ordinary processes of cooking. In any case their spores would almost certainly retain their vitality. Fortunately the occurrence of the spores of such bacteria as those of anthrax or tetanus in meat is very exceptional.

Changes in the Juices of Meat on Cooking.—According to Strohmer an approximate idea of the highest temperature reached within the interior of the flesh may be formed from the appearance of the juice pressed from the cooked meat. He states that if this is a turbid liquid the temperature did not exceed 56° C. If it is clear red the temperature was probably between 50° and 60° C., but not exceeding 65° C. Between 70° and 72° C. the colour of the juice changes to brownish red, and between 75° and 80° C. to yellow.

The Public Sterilisation of Infected Flesh in Germany.—Flesh containing only a few cysticerci, or infected with certain diseases, such as swine plague, swine erysipelas, etc., is allowed to be sold in Germany after having been thoroughly disinfected by cooking, under police supervision. For the sale of such meat, and of flesh which has been passed as of inferior quality, though not dangerous to health, institutions, known as *Freibänke*, have been established in connection with the local meat inspection in many of the towns, especially in the South of Germany. Such meat stamped by the *Freibank* is sold at a very cheap rate, and is largely used by the poorer classes.

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* Ostertag, *Handbuch der Fleischbeschau*, p. 548.

The cooking is so arranged that the meat is thoroughly heated throughout. The flesh is divided into thin strips, which are boiled for two or three hours, or until the interior becomes grey. In this process, as ordinarily carried out, there is a considerable loss in nutritive value, and in order to obviate this, sterilisation by means of steam under pressure has been introduced in many places. In Rohrbeck's steam steriliser, constructed on this principle, every part of the meat is brought to a temperature of at least 100° C., while the meat juices and the flavour are retained to a much greater extent than is otherwise possible.

CHAPTER XI.

POISONOUS FLESH.

FLESH may sometimes be rendered injurious by contamination with some drug such as chloride of lime, phenol, etc., but, apart from such cases, it may have inherent toxic properties, which may be derived—(1) from some injurious substance eaten by the animal; (2) from poisonous products secreted by the cells of the living animal; (3) from pathogenic bacteria or bacterial products in the living animal; or (4) from *post-mortem* alteration of the flesh by bacteria.

Flesh rendered Poisonous by the Food of the Animal.—Numerous instances are on record showing that flesh which is ordinarily wholesome may become more or less injurious from the food which the animal has eaten shortly before being killed. According to Letheby,[*] the flesh of hares which have fed upon the *Rhododendron Chrysanthemum* has caused illness, and similarly in Pennsylvania and Philadelphia, pheasants which have eaten the buds of the laurel (*Calmia latifolia*) are unwholesome. In fact, Letheby attributes many of the illnesses which have occurred after eating prairie birds imported into this country from America to the nature of the food eaten by the bird. Sometimes in Australia the flesh of sheep acquires poisonous properties from the animals having fed upon the lotus, wild melon, and wild cucumber, the general effects being pains in the limbs, prostration, and sickness. The animals themselves are occasionally, but not invariably, poisoned by their food.

Possibly some of the cases of shell-fish poisoning which happen from time to time are to be attributed to this cause. And it is interesting to note in this connection that the *Maletta venenosa*, a poisonous tropical fish, is said to be venomous only at the times when the sea is covered with a green monad on which it feeds (Letheby). In 1842 a whole family in Toulouse were poisoned by eating a dish of snails collected from a poisonous shrub, *Coriaria myrtifolia*. Guenther[†] states that the poisonous nature of the flesh of most, if not all, of the poisonous fish of the tropics is derived from their food, which consists of medusæ, corals, or decomposing substances.

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[*] *On Foods*, p. 221.

[†] *The Study of Fishes*, p. 189.

Poisons.—Of inorganic poisonous substances taken by the animal, phosphorus is the only one known to produce more than local effects. In phosphorus poisoning the general symptoms are extravasation of blood, alteration of the tissues, and fatty degeneration. The blood is altered in appearance, and the flesh becomes phosphorescent in the dark.* Wally considers that, with the exception of phosphorus, "inorganic poisons are never absorbed in sufficient quantity to render the flesh of the animal nocuous."

The different instances mentioned above of flesh made poisonous by the food of the animal show that certain organic poisons, apparently of an alkaloidal or glucosidal nature, can produce general symptoms, and this is probably the case with many other organic poisons.

Flesh Poisonous from Products elaborated by the Cells of the Living Animal.—*Formation of Leucomaines and Toxines.*—In 1882 Gautier showed that just as toxic products (ptomaines and toxines) are secreted by certain bacteria, so by a sort of enzymic action in the living cells the proteids or other nitrogenous compounds are normally broken down into less complex bodies, being finally transformed into urea, amides, hydrocarbons, carbon dioxide, bases, etc. To the basic substances, which are closely allied to ptomaines in many of their reactions and properties, he gave the name of *leucomaines*,† or physiological alkaloids. The process of their formation is primarily a direct hydration, and is regarded by Gautier as an anaërobic fermentation. Most of the leucomaines thus formed are harmless, or only slightly poisonous, but some are extremely toxic, such as neurine and choline.

Under certain circumstances leucomaines, or other decomposition products of proteids, may be of such a nature, or produced in such quantity and insufficiently eliminated from the system, as to cause auto-infection. An interesting illustration of this is afforded by the experiments of Professor Mosso of Turin,‡ who found that the illness caused by over-fatigue is due to the absorption of certain substances into the blood, and that these substances when injected into healthy animals produce the same symptoms.

The presence of leucomaines in excess, or of toxines derived from the proteids, is probably the cause of the illness sometimes produced by eating the flesh of over-hunted game or of over-driven cattle. Liebig, in his *Letters on Chemistry*, mentions a case in which the flesh of a roebuck, which had struggled violently after having been caught in a snare, gave rise to symptoms of poisoning. According to Gautier,[*] pigs have been fatally poisoned through being fed upon the flesh of a horse which had died during its struggles when being broken in, and, like Liebig, he has known of cases of human poisoning by the flesh of roebucks which had died in a state of terror or exhaustion.

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* Andrews, *Handbook of Public Health*, p. 20.

† λευκωμα = white of egg.

‡ *Lancet*, 1887, p. 1295.

Gautier[†] has also confirmed Landi's statement that when muscle, the cells of which are still living, is taken from an animal and protected from the influence of putrefactive bacteria, the action of the cellular protoplasm continues, and by a sort of anaërobic decomposition causes an increase in the extractives and toxic bases of meat, especially of those belonging to kreatinic and neurinic groups. Simultaneously there is a diminution in the proteids, and the glycogen disappears, but the fat is not appreciably affected.

Roger[‡] considers that the toxicity of the extract of normal muscle is to be attributed to toxines of a proteid nature rather than to basic bodies, which are only moderately poisonous. By removing the crystallisable substances by dialysis, he obtained a residue of an albuminous nature, which on injection into rabbits produced symptoms of exhaustion, somnolence, diarrhœa, and death without, or attended only by slight, convulsions. The fact that the extract after being heated to 100° C. did not cause these results was regarded as proof that the leucomaines are not the poisonous substances.

Leucomaines which are also known as Ptomaines.—Among the basic substances which have been found both in the products of bacterial putrefaction and among the substances elaborated by living cells, probably by the decomposition of lecithins, the following may be mentioned:—

Choline [$C_5H_{15}NO_2$], which occurs normally in the blood, muscular tissue, and glands of the ox and other animals. It resembles neurine in its toxic action, but is weaker.

Neurine [$C_5H_{13}NO_3$], which usually accompanies choline in traces, and is found in the brain and nerves. It is very toxic, and is regarded by Gautier as the probable cause of the roe of certain fish becoming poisonous at the spawning season.

Betaine [$C_5H_{11}NO_2$], which is normally present in many animals, notably the mussel.

Trimethylamine [$(CH_3)_3N$], which occurs in blood.

Neuridine [$C_5H_{14}N_2$], found in the yelk of egg and in fresh human brain.

Cadaverine [$C_5H_{14}N_2$], isolated in traces from fresh pancreas.

Gerontine [$C_5H_{14}N_2$], found in the liver of an old dog.

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[*] *Les Toxines*, 1896, p. 438.

[†] *Ibid.*, p. 456.

[‡] Gautier, *loc. cit.*, p. 455.

Poisonous Fish.—The following table of fish, certain species of which are known to be poisonous, either invariably or at certain seasons of the year, or after eating certain food, is given by O. W. Andrews[*]:—

Acanthopterygii or Spiny-rayed fishes, including *Sparidæ* (sea-breams); *Squamipinnes* (coral fishes); *Sphyrænidæ* (barracudas); *Scombridæ* (mackerel); *Carangidæ* (horse-mackerel); *Acronuridæ* (sturgeons), and *Atherinidæ*.

Pharyngognathi, including *Labridæ* (wrasses).

Physostomi, including *Siluridæ* (cat-fish); *Clupeidæ* (herrings).

Plectognathi, including *Sclerodermi*, *e.g.*, *Balistes* and *Ostracion*; *Gymnodontes* (*Diodon*, *Triodon*, and *Tetrodon*).

Of the bream family (*Sparidæ*) the Spanish bream (*Pagelus erythrinus*) is met with off the shores of New Caledonia and New Hebrides. Its poisonous properties are possibly due to the nature of its food. The *Lethrinus mambo* is another member of the same family, and is found in the same waters. Its flesh is said to be innocuous when young, but to be very poisonous when full grown.

Among the coral-fish the *Heniochus macroleptidotus* is distinguished for the brilliance of its colouring and its poisonous properties. It is found in the neighbourhood of coral-reefs and is carnivorous.

The barracudas (*Sphyræna barracuda*) are found in the West Indies, and are usually poisonous. They are large fish, often 8 feet long and 40 lbs. in weight.

The *Caranx fallax*, which is a member of the horse-mackerel family, is said to be wholesome when young, but poisonous when full grown. It is met with in Australian waters.

Different varieties of wrasse are known as parrot-fish from their brilliant colour, and appear to be poisonous from the nature of their food.

The cat-fish (*Siluridæ*) are usually found in fresh water, and only those varieties which enter the sea are regarded as poisonous. Their skins are smooth and without scales.

According to Guenther[†] the flesh of certain members of the herring family, such as *Clupea thryssa* and *Clupea venenosa*, is always poisonous. *Clupea thryssa* (the yellow-billed sprat) is exceedingly poisonous, and has been known to cause death before being actually swallowed.

Tetrodon and *Diodon*, known as 'globe-fishes,' are covered with spines, and have the power of distending their bodies with air into a globular form, and floating on the surface of the water with the underside uppermost and spines protruding. Guenther states that some of the species are always poisonous.

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[*] *Handbook of Public Health*, 1898, p. 51.

[†] *The Study of Fishes.*

Balistes, or 'file-fishes,' are so called from the file-like edge of the dorsal fin. They feed on coral and molluscs, from which they probably derive their poisonous properties.

The *Ostracion*, or 'trunk-fish,' is protected by a covering of bone-like plates. Like the preceding fish, it is found off the American coast and in Indian waters, and is universally regarded as poisonous.

Many of the smaller varieties of these poisonous fish are eaten by larger fish, such as different species of dolphin, conger-eel, etc., and cause the flesh of these to be also poisonous for some time afterwards. In some fish a poisonous substance appears to be secreted only at certain times of the year, as, for instance, in the case of the pike and the turbot, whose roe produces violent diarrhœa when eaten during the breeding season.[*]

According to Letheby the general symptoms caused by eating poisonous fish such as these, are either irritation of the stomach and intestines, with choleraic symptoms, or rapid prostration and convulsions.

Flesh rendered Poisonous by Bacteria in the Living Animal.—In addition to certain definite diseases, such as tuberculosis, which may sometimes be communicated through the presence of the specific bacilli or their products in the flesh, there have been numerous obscure cases of poisoning, the symptoms of which resembled, to some extent, those of ptomaine poisoning, although there was no sign of putrefaction in the meat. In some of these cases it is not improbable that ptomaines may actually have been present, and have contributed to the result, since it has been proved that these bases may be formed in cultivations by bacteria other than those usually associated with putrefaction. Thus putrescine has been isolated from cultivations of the *Bacillus coli communis*, cadaverine from cultivations of Koch's comma bacillus and Finkler and Prior's bacillus, and methyl-guanidine from the substances elaborated by the bacillus of mouse septicæmia.

Bollinger[†] considers that septicæmia and pyæmia must be regarded as the causes of many cases of poisoning, and he considers them as of almost more importance than any other disease, owing to their frequent occurrence. During the four years preceding 1880 he had under his notice eleven cases of wholesale poisoning, and 1600 cases of individual illness, which he considered were undoubtedly the result of septicæmia or pyæmia.

In 1874, in Bregenz, fifty-one people were poisoned by the flesh of a cow which had been slaughtered on account of septic injuries received during calving. A similar case occurred in 1876 in Bavaria, in which twenty two persons were made ill with choleraic symptoms. The cooked flesh and cooked sausages made from it were also injurious. In another instance seven people were poisoned by beef from a young cow which had been infected with puerperal sepsis before being killed. After four days the flesh showed marked signs of decomposition. In Ostertag's * experience there were 1500 cases of illness of this nature, principally in Germany, during the twelve years preceding 1892.

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[*] Guenther, *loc. cit.*, p. 189.

[†] Ostertag, *Handbuch der Fleischbeschau*, p. 469.

From these and similar cases, which might be cited *ad infinitum*, there can be but little doubt that a septic condition in the animal is a frequent cause of its flesh having poisonous properties.

Fish Cholera.—This is a disease which is epidemic among sturgeon and other fish. Its cause was investigated by Sieber-Schoumow,† who found in the stomach and intestines of the fish a motile and anaërobic bacillus, *B. piscicidus agilis*, which, on inoculation, produced the disease in healthy fishes. From the sterilised cultivation a very toxic base was separated, which he regarded as contributing to the disorders produced by fish which are normally wholesome, but become poisonous when attacked by this and similar bacteria.

Fischer and Eber isolated from the blood of a carp which had been killed by the impurity of the water a bacillus which was exceedingly toxic to warm- or cold-blooded animals, and which elaborated a poisonous toxine. This, unlike the ptomaine of fish cholera, was destroyed by boiling.

Mussel Poisoning.—Severe illness is sometimes produced by eating mussels, the principal symptoms being vomiting, diarrhœa, difficulty in breathing, feeble pulse, prostration, a rash all over the body, dilation of the pupil of the eye, and sometimes swollen tongue and throat. In 1885 there was an epidemic of mussel poisoning in Wilhelmshaven, many of the cases proving fatal in the course of three or four hours. Some of the victims had not eaten more that five or six of the mussels. König states that poisonous mussels are usually of a brighter yellow colour, but those of darker colour have also been known to cause the same symptoms.

The life conditions of the mollusc appear to have a considerable influence on its wholesomeness, for Virchow and Schmidtmann found that when poisonous mussels were left in pure sea-water they became harmless in the course of a month. Similarly

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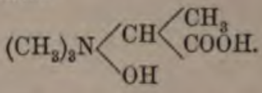
* *Handbuch der Fleischbeschau*, p. 475.

† *Arch. Sciences biol. de St. Petersburg*, 1894, iii. p. 241.

M. Wolff and König * found that mussels placed in the stagnant water of the harbour became poisonous in two or three weeks, while poisonous mussels placed in the neighbourhood of a sluice where the water was frequently changed became harmless again. The mussels collected in January and February were more poisonous than those gathered in November and December.

From Wolff's * investigations the poison seems to be chiefly developed in the liver, while the foot, gills, mantle, and eggs are non-poisonous. Schmidtmann † considers that the poison is caused by a definite disease, probably bacterial and communicable.

Mytilotoxine. — Brieger ‡ isolated from poisonous mussels a ptomaine or leucomaine to which he gave the name of *mytilotoxine*. It was accompanied by large quantities of *betaine*, which was also found in non-poisonous mussels. The constitution of mytilotoxine is uncertain, but it may be regarded as a methyl derivative of betaine with the formula



$$(CH_3)_3N\left\langle\begin{matrix}CH\left\langle\begin{matrix}CH_3\\COOH.\end{matrix}\right.\\OH\end{matrix}\right.$$

Possibly it is formed by a diseased condition of the animal from the betaine normally present or by the action of pathogenic bacteria derived from the water.

The method adopted by Brieger for its isolation is described on page 315. It was not found among the products of the putrefaction of ordinary non-poisonous mussels. The free base is an unstable resinous body with a disagreeable odour. It is extremely toxic, and the least traces of its hydrochloride, when injected into animals, produce all the symptoms of mussel-poisoning. Free mytilotoxine rapidly loses its poisonous properties on heating, and on dry distillation yields large quantities of trimethylamine.

Flesh rendered Poisonous by the Action of Bacteria on the Dead Flesh.—It had long been known that an aqueous extract of decomposed animal matters had toxic properties, but it was not until 1855 that the Danish chemist Panum showed that the poison was of a chemical nature, and probably contained several active principles.

His results were confirmed by Bergmann, Müller, and other chemists, especially in Germany, but the chemical nature of the toxine was not determined. In 1869 Sonnenschein and Zülzer extracted from flesh which had been left to decompose for five or six weeks traces of a crystalline basic substance, which gave the reactions of alkaloids, and had physiological properties resembling those of atropine. In the following year Selmi found that substances of an alkaloidal nature were normally present in the stomach of a dead animal before and after putrefaction; in 1874 he definitely announced that basic substances resembling the vegetable alkaloids were formed during putrefaction, and gave them the name of 'ptomaines' (πτωμα = dead body); and finally, in 1877, came to the conclusion that these substances were bacterial products.

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* König, *Nahr. Genussm.*, ii. p. 103.

† Quoted by Gautier, *Les Toxines*, p. 135.

‡ *Virchow's Archiv*, 1889, cxv. p. 483.

The first ptomaine isolated as a pure chemical substance was collidine, which was extracted by Nencki from a putrified infusion of gelatin. Since then a large number of well-defined ptomaines have been isolated by other workers in this field, among whom may be mentioned Gautier and Etard, Pouchet, Salkowski, and especially Brieger.

Summary of the Principal Ptomaines.—The principal bases which have been separated from decomposing flesh are given in the subjoined table, in which Gautier's scheme of classification has been adopted.

Monamines of the Fatty Acid Series.

Trimethylamine. $(CH_3)_3N$. Herring pickle.
Di-ethylamine. $(C_2H_5)_2NH$. Putrid meat extract.
Tri-ethylamine. $(C_2H_5)_3N$. Decomposed cod-fish.
Propylamine. $C_3H_7NH_2$. Decomposing cod-liver.
Butylamine. $C_4H_9NH_2$. Do. do.
Amylamine. $C_5H_{11}NH_2$. Cod-liver oil.

Diamines of the Fatty Acid Series.

Putrescine, or Tetramethylene-diamine. $C_4H_{12}N_2$. Putrid horseflesh.
Cadaverine, or Pentamethlyene-diamine. $C_5H_{14}N_2$. Putrid fish and blood.
Neuridine. $C_5H_{14}N_2$. Putrid meat, albumin, gelatin.
Saprine. $C_5H_{14}N_2$. Decomposed flesh.

Guanidines.

Methylguanidine. $C_2H_7N_3$. Putrid horseflesh and beef.

Aromatic Ptomaines, free from Oxygen.

Collidine. $C_8H_{11}N$. Putrid fish and putrid gelatin.
Parvoline. $C_9H_{13}N$. Putrid horseflesh after several months.
Corindine. $C_{10}H_{15}N$. Putrid cuttle-fish.
Di-hydrocollidine. $C_8H_{13}N$. Putrid fish and horseflesh.

Oxygenated Ptomaines.

Neurine. $C_5H_{13}NO$. Putrid meat on fifth or sixth day.
Choline. $C_5H_{15}NO_2$. Accompanies neurine.
Muscarine. $C_5H_{15}NO_3$. Putrid fish.
Betaine. $C_5H_{11}NO_2$. In mussels (leucomaine).

Homopiperidinic Acid. $C_5H_{11}NO_2$. Decomposition of meat fibrin.

Mytilotoxine. $C_6H_{15}NO_2$. In poisonous mussels (? leucomaine, *cf.* p. 222).

Mydatoxine. $C_6H_{13}NO_2$. Putrid horseflesh after nine to fifteen months.

Gadinene. $C_7H_{17}NO_2$.
Methylgadinene. $C_{18}H_{17}NO_2$. } Putrid fish, especially cod.

Unnamed Base of Brieger. $C_7H_{17}NO_2$. Accompanies mydatoxine.

Aromatic Oxygenated Bases.

Tyrosamines. C_7H_9NO; $C_8H_{11}NO$; $C_9H_{13}NO$. Decomposing cod-liver.

Mydine. $C_8H_{11}NO$. Decomposing human flesh.

Symptoms of Ptomaine Poisoning.—The usual symptoms of ptomaine poisoning are dilation of the pupil of the eye, followed by its contraction, feeble pulse, slow respiration, fever, loss of muscular contractibility, stupor, convulsions, and death. The loss of the power of contracting the muscles, even under electrical stimulus, is remarkable, and is a characteristic symptom of poisoning by muscarine, a ptomaine which is found both in putrefying flesh and in poisonous mushrooms.

The ptomaines vary considerably in their physiological action, some being quite inert, while others are fatal even in small doses. The symptoms of flesh poisoning probably vary in kind and degree with the nature and quantity of the bases present, some of which may modify to a greater or less extent the action of the others.

Of the monamines formed during the putrefaction of flesh, the *methylamines* and *ethylamines* are only moderately poisonous, tending to produce fever; *butylamine* in large doses produces convulsions and muscular paralysis; and *amylamine*, which is very poisonous, causes dilation of the pupils of the eye and convulsions.

The diamines (*putrescine*, *cadaverine*, *neuridine*, and *saprine*) are either physiologically inert or at most only slightly poisonous. *Cadaverine* is said to produce inflammation of the mucous membrane.

Methyl-guanidine, which may be taken as representative of the guanidine ptomaines, is exceedingly toxic. It produces dilation of the pupils, convulsions, and death within twenty minutes, when injected into a small animal.

Of the aromatic non-oxygenated ptomaines, *collidine*, *parvoline*, *corindine*, and *di-hydrocollidine* are all extremely poisonous. *Corindine* resembles curare in its effects, causing paralysis.

Di-hydrocollidine produces torpor, muscular paralysis, and convulsions.

Of the better-known oxygenated ptomaines, *neurine* produces salivation, contraction of the pupil of the eye, sudden convulsions, and death. *Choline* resembles neurine in its physiological action, but is much weaker. *Muscarine* is exceedingly toxic, and in small doses produces salivation, contraction of the pupil of the eye, diarrhœa, convulsions, and death. The action of atropine is antagonistic to the three preceding ptomaines, and is used as an antidote. *Betaine* is non-poisonous. *Mydatoxine* is moderately poisonous. In large doses it causes diarrhœa, redness of the eyes, convulsions, and death. *Gadinene* is not very poisonous, but *methylgadinene* in sufficiently large doses produces symptoms of paralysis. An unnamed base of Brieger ($C_7H_{17}NO_2$), which was found accompanying *mydatoxine* in putrid horseflesh, has poisonous properties resembling those of curare.

Botulism or **Sausage Poisoning.** — Like the attacks of trichinosis, cases of *botulism* have been most frequent in those parts of Germany where, as in Saxony, raw ham and raw sausage are most widely eaten. Sometimes the poisoning has been wholesale, as in the Chemnitz cases, where, in 1879, 241 individuals were poisoned by *Mettwurst*, and where, seven years afterwards, 160 persons were poisoned in the same way. Ostertag[*] mentions smaller outbreaks of the same kind since 1886, as, for instance, in Dresden (11), in Gerbstadt (over 50), and in Gera (30).

The characteristic symptoms of pure botulism appear after a period of incubation of from eighteen to forty-eight hours. They commence with a feeling of uneasiness and pressure in the stomach, followed by vomiting and, occasionally, diarrhœa, with faintness, disturbance of the vision, muscular flaccidity, and collapse. When the case ends fatally death results in from four to eight days. When the toxine of *B. botulinus* is the sole contributing cause of the illness, fever and mental disturbances are not among the symptoms. The mortality is very high, and, according to Senkpiehl, out of 412 cases recorded between 1789 and 1886 there were 165 deaths.

Eber regarded both sausage poisons and ptomaines as toxigenic substances, not toxines. Under the term 'toxigenes' he grouped those chemical products which, on injection into an animal, are not poisonous until they have been modified by the vital activity of the cells. He compared them with certain inorganic substances, such as sodium iodide, which, when injected into an animal, produce no ill effects for some six or eight hours.

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[*] *Loc. cit.*, p. 502. Schneidemühl, *Cent. f. Bakt.*, 1898, p. 577.

The origin of the poison remained unexplained for years, although it had long been recognised as distinct from that derived from ordinary putrefaction. Hilger[*] was the first to isolate from the intestines of six persons who had died from sausage poisoning a semi-fluid substance with properties resembling those of curare, and Tamba found a similar substance in liver sausage exposed to the air.

Haupt believed that the disease was produced by the decomposition products formed by *B. proteus mirabilis*, but Ostertag pointed out that the symptoms of botulism did not agree with those produced by the inoculation of cultivations of that micro-organism.

In 1895 van Ermengem isolated, from the body of a victim to sausage poisoning, an anaërobic bacillus, the cultivations of which produced the same symptoms. The characteristics of this bacillus, which was never found in putrefying substances, are given on page 278.

Brieger and Kempner[†] have recently isolated from pure cultivation of *B. botulinus* a toxine which they regard as closely related in chemical composition to the toxines of diphtheria and tetanus. The dried toxine kept well, and was found to produce all the symptoms of sausage poisoning. From putrefying liquids or flesh no poisonous products with the same pathogenic properties could be isolated. The symptoms caused by products of the *coli* species, to which flesh poisoning has often been attributed, had no specific effects, and the cultivations of Gaertner's *B. enteritidis* produced only very slight symptoms.

The toxine of *B. botulinus* is rendered inactive by being heated to 60° or 70° C.

Kempner[‡] found that by injecting gradually-increasing doses of the toxine into goats the animals were rendered immune, and that guinea-pigs treated with the blood serum of the immune goats were made capable of withstanding a dose of the toxine 100,000 greater than one which, under other circumstances, would have been fatal.

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[*] König, *Nahr. Genussm.*, ii. p. 103.

[†] *Cent. f. Bakt.*, 1898, p. 619.

[‡] *Zeit. f. Hyg.*, 1897, xxvi. p. 481.

CHAPTER XII.

THE ANIMAL PARASITES OF FLESH.

To give even a brief account of all the internal parasites found in different animals would require much more space than can be spared for it here, so that, with a few exceptions, the various organisms described in this chapter will be limited to those connected directly or indirectly with flesh considered as human food.

Internal parasites, or *Entozoa*, may be defined as lower organisms, which either in an immature or adult condition inhabit the tissues or canals of different organs, or of the muscle or skin of higher animals, either in a free or encysted state.

They may be grouped into three main divisions:—

Protozoa.—Low organisms whose bodies are composed of contractile tissue and are usually without definite structure.

Infusoria.—Microscopic organisms provided with mouths, or at least suction tubes, such as, for example, *Cercomonas intestinalis*, found by Davaine in the excreta of a cholera patient.

Helminthia, or true intestinal worms.

Of the first class the parasites in the sub-order of *Sporozoa* are of primary importance in the examination of flesh and flesh products, and of the third class the three orders—*Cestoda*, or tapeworms; *trematoda*, or flukes; and *nematoda*, or round worms—likewise require special attention.

SPOROZOA.

These form a class in the sub-kingdom of *Protozoa*. They are unicellular organisms devoid of definite progressive organs (*pseudopodia* or *cilia*), but often provided with an organ of attachment. Their food is absorbed by endosmosis, and their whole lives are spent as parasites. When adult they reproduce their species by the formation of spores (*psorospermiæ*) in their interior. Within these spores are formed small sickle-shaped bodies, from which are developed new parasites. The organisms of this class most commonly met with in the examination of flesh are the so-called 'psorosperm saccules,' the formations known as '*Miescher's tubes,*' and the *coccidia* with which rabbits are often affected.

'Psorosperm Saccules.'

These sporozoa, first discovered by J. Müller, are found in the muscle and on the skin and gills of fishes, and when visible to the naked eye have the appearance of small white specks. They vary very considerably in size, some being microscopic, while others are several millimetres in diameter.

Miescher's Tubes (*Synchitrium Miescherianum*).

These curious formations derive their name from Miescher, who first noted their occurrence in the muscles of a mouse. They have since been found to be widely distributed, and are frequently met with in the flesh of the pig, ox, sheep, deer, and other animals. In the normally extended muscle they have the appearance shown in the accompanying figures, but when the muscles are freed from their insertions and the fibres contract, the tubes become broader and shorter. They have a thick exterior wall of cuticle, and contain a tough matrix of protoplasm, in which are small bean-shaped granules, 0·01 mm. in diameter. In the younger and smaller tubes (0·7 to 1 mm.) transparent balls (probably spores) may often be observed.

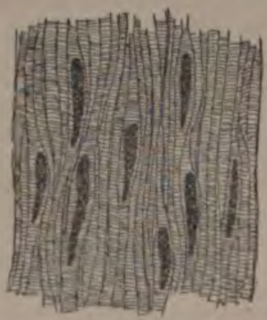
FIG. 19.—Preparation of muscle containing Miescher's Tubes. (*Leuckart.*)

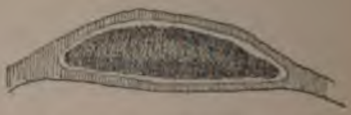
FIG. 20.—A single Miescher Tube in a muscle fibre. More highly magnified. (*Leuckart.*)

Miescher's tubes are usually classed among the sporozoa, but there is some doubt on the point, since no movements have been observed in the stage of development. Perroncito, in his experiments on the action of heat on various parasites (p. 248), found that these organisms never showed any signs of movement as the temperature rose. On the other hand, Leuckart * states that by feeding a pig which was free from the tubes on flesh containing them, he succeeded in infecting the animal, and its muscles were subsequently found to be full of tubes. So far as is known these organisms are without pathological significance.

They can be readily stained by means of carmine or methylene blue. Marpmann recommends a counter-stain composed of phloxin red 1 part, and methylene blue 1 part, in dilute alcohol. The section from the flesh is pressed between cover glass and dipped in the stain for ten minutes, then washed with alcohol and water, and examined under the microscope. In this way the organism is stained blue and the muscular fibres red.

Coccidium Oviforme.

This organism may be taken as a representative example of the *coccidia.* It has been found in invertebrata (snail, etc.), in various mammalia, and in man, but it is most frequently met with in rabbits, where it produces what is known as the 'coccidial disease.' The livers of the infected rabbits are often found permeated with white nodules, some of which attain the size of a nut; and on section a cheesy mass exudes, which contains innumerable numbers of the coccidia. Sometimes the disease becomes epidemic, and the whole of the rabbits in a warren become infected. The secretions of the liver and bile are interfered with, and the tissue of the glands destroyed. The animals become thin and sick, then shortness of breath and convulsions ensue, and finally death.

These sporozoa are also known as 'egg-shaped psorosperms,' a name which rightly belongs only to the spores formed within them. The coccidia vary in size from 0·35 to 0·37 mm. in length, by 0·015 to 0·02 mm. in breadth.

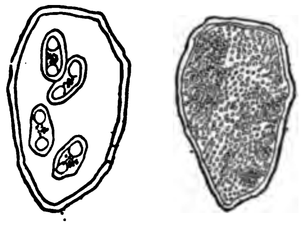

FIG. 21.—Coccidia in the Liver of a Rabbit, one showing psorosperms in interior. × 660. (After *Leuckart.*)

In the earliest stage of their life history these and other coccidia are found in a free state in the epithelial cells, but towards the end of the period of growth they become enveloped in a firm shell, and leave their resting-place and generally their original host. The granular protoplasm is condensed into a mass in the centre of the capsule, and spores are formed, each containing a granular ball and a sickle-shaped body. In the case of the *Coccidium oviforme* the spores are only produced after expulsion with the fæces from the body of the host, and the further development proceeds in moist surroundings outside. The spores are round or elliptical, and have a rather thin wall. According to Leuckart * they are invariably four in number.

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* *Human Parasites,* p. 199.

THE CESTODA.

This class of parasites includes the tapeworms and allied organisms. They are flat worms devoid of mouth or alimentary canal. The 'head' or nurse (*scolex*) is provided with two or more suckers, and in many cases with curved hooks of attachment, by means of which it fastens itself on to the intestinal membrane of its host, which is usually a vertebrate animal. Here it increases joint by joint, forming a long ribbon-like colony, which remains attached to the head for a considerable period. The individual sexual segments (*proglottides*) increase in size, and become more mature or 'ripe' as they become further removed from the head by the formation of other segments. The ripe joints are expelled from the body of the host, and the embryo which each contains becomes a bladder-worm (*Cysticercus* or *Cysticercoid*), usually in the muscles or organs of another animal or intermediate host. Here it remains quiescent until introduced into the intestine of a subsequent host, where the head of the larva attaches itself to the membrane, and a new tapeworm is produced.

CLASSIFICATION OF TAPEWORMS.

Leuckart † gives the following scheme of classification of representative *Cestoda* :—

FAMILY: TÆNIADÆ.

DIVISION I.

Cystici (Cystic tapeworms).
  Sub-genus.—Cystotænia (Leuckart).
    1. Tænia saginata.
    2. T. solium.
    3. T. acanthotrias.
    4. T. marginata.
  Sub-genus.—Echinococcifer (Weinland).
    5. T. echinococcus.

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* *Loc. cit.*, p. 202.

† *Human Parasites*, p. 390.

DIVISION II.

Cystoidei (Ordinary tapeworms).

Sub-genus.—Hymenolepis.
- 6. T. nana.
- 7. T. flavo-punctata.

Sub-genus.—?
- 8. T. madagascariensis.

Sub-genus.—Dipylidium.
- 9. T. cucumerina.

FAMILY: BOTHRIOCEPHALIDÆ.

Genus.—**Bothriocephalus.**
1. B. latus.
2. B. cristatus.
3. B. cordatus.
4. B. liguloides.

USUAL HOSTS OF SOME TAPEWORMS.

The following table shows the hosts of some of the better-known bladder-worms and their related tapeworms:—

Larva.	Host.	Tapeworm.	Host.
Cysticercus cellulosæ,	Swine, dog, bear, deer, rat, ape, man.	*Tænia solium*,	Man.
C. bovis,	Ox, goat (experiment), giraffe.	*T. saginata*,	Man.
C. acanthotrias,	Man, ox (probable).	*T. acanthotrias*,	Not known, probably man.
C. tenuicollis,	Swine, ruminants.	*T. marginata*,	Dog, wolf.
Echinococcus hominis,	Ox, sheep, swine, man.	*T. echinococcus*,	Dog.
C. fasciolaris,	Mouse.	*T. crassicollis*,	Cat.
C. pisiformis,	Hare, rabbit.	*T. serrata*,	Dog.
Cœnurus cerebralis,	Sheep, ox, squirrel.	*T. cœnurus*,	Dog.
Cysticercus T. cucumerinæ,	Dog-louse (Trichodectes canis).	*T. cucumerina*,	Dog, cat, man.
Larva of Bothriocephalus latus,	Pike and other river fish.	*Bothriocephalus latus*,	Man, cat (experimentally).
,, *B. cordatus*,	Probably marine fish.	*B. cordatus*,	Dog, man.
Cysticercus ovis,	Sheep.	(?) *T. tenella*,	Man (?).

Family I.—The Tæniadæ.—The tapeworms in this branch of the Cestoda have small spherical or pear-shaped heads supported and moved by a muscular proboscis, *the rostellum*, and provided with four suckers for attachment, and usually with one or more circlets of hooks. The division of the individual segments is well marked, and each retains its enclosed eggs until the proglottis itself is destroyed.

The Tæniadæ fall naturally into two divisions:—I. Cystic tapeworms, which at a certain stage of their growth as larvæ form bladder-like cysts containing liquid (*hydatids*). II. Ordinary tapeworms, in which the embryonic body is solid or nearly solid.

I.—CYSTIC TAPEWORMS.

These can be further subdivided into two more groups. A, "Those in which the head arises within the embryonic bladder," and B, "Those whose heads are budded off from special brood capsules attached to the inner surface of the bladder."* With the exception of *T. saginata* and *T. solium*, all the tapeworms of Group A are found in carnivorous animals. *T. echinococcus* is representative of group B.

GROUP A.

Tænia saginata.—*General Characteristics.*—This tapeworm, also known as *T. medio-canellata*, *T. lata*, and *T. dentata*, is common throughout Europe, Asia, and Africa, and is the tapeworm most frequently met with in Bavaria, Hungary, Italy, and Turkey. When full-grown it is about 4 metres in length in its contracted state, and from 7 to 8 metres when extended. It usually has from 1200 to 1300 segments, of which from 150 to 200 are 'ripe' proglottides. The middle segments measure from 12 to 14 mm., and those of the neck seldom less than 1 to 1·5 mm. The head is large (1·5 to 2 mm.), and has a flattened crown, in which is a hollow depression.

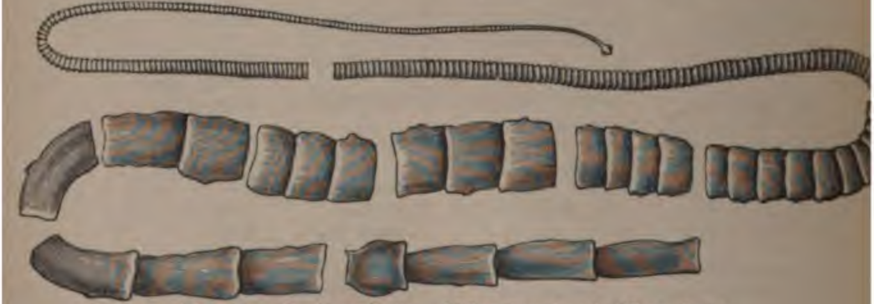

FIG. 22.—Tænia saginata. (After *Leuckart.*) Natural size.

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* Leuckart.

It has four suckers, but is devoid of hooks (figs. 23 and 31). Each ripe proglottis is capable of holding about 3500 eggs (Leuckart). The entire colony of proglottides is renewed every three months, and Cobbold* estimates that a single tapeworm can thus distribute annually twelve million eggs.

Cysticercus Bovis.—The bladder-worm of *T. saginata* is found almost exclusively in the muscles of the ox, cow, and calf, and most of the attempts to rear it in other animals have been unsuccessful. Zenker, however, claims to have succeeded in the case of the goat, and it has also been known to occur in the giraffe. It is most frequently met with in the facial muscles, and then in those of the heart and tongue, while other muscles (neck, breast, etc.) are comparatively free.

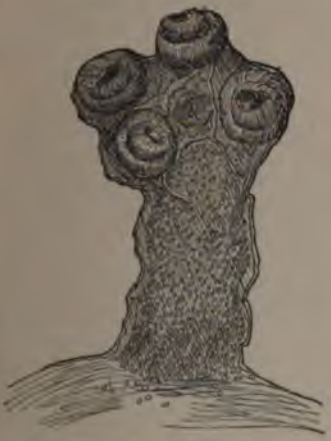

FIG. 23.—Head of Cysticercus of *T. saginata*. × 25. (After *Leuckart.*)

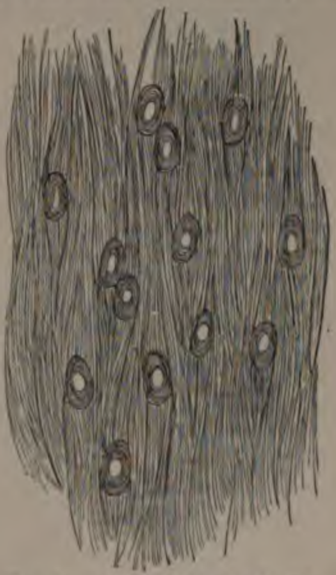

FIG. 24.—'Measles' in Beef. Two-thirds of the natural size. (*E. Mitchell.*)

It originates by the animal swallowing a 'ripe' *proglottis* (or its eggs) which has been expelled from the body of the host of the parent tapeworm. In about eighteen weeks the eggs develop into completely formed bladder-worms, which, however, continue to grow for about ten weeks. When full grown each envelops itself in a long oval cyst from 3 to 5 mm. in diameter. This contains a transparent fluid, within which the retracted head of the worm can be distinguished. The head resembles that of the sexually complete tapeworm in being provided with suckers, but no hooks. It remains quiescent in the cystic state until the animal dies or is killed and the flesh eaten by man, when it attaches itself to the intestines by means of the suckers, and completes its development.

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* *Parasites of Man*, p. 12.

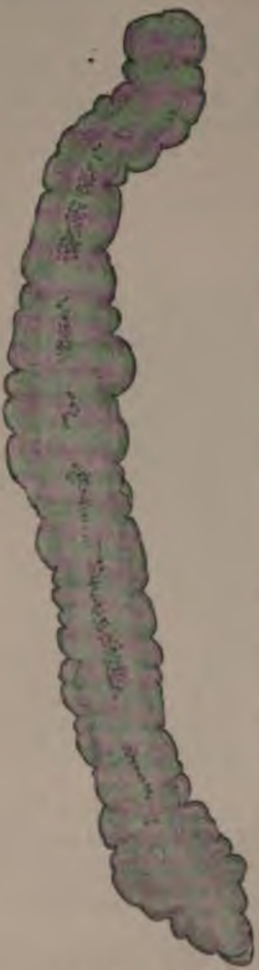

FIG. 25.—Section of free Proglottis of Tapeworm (*Tænia solium*). ×18. (After *R. M. Prideaux.*)

'Measles' in Beef.—The muscle containing the encysted bladder-worms, which resemble little white knots, has often been termed 'measly' from its appearance (fig. 24).

There is usually a thick well-developed connective tissue around the cysts, and not infrequently the worm is found dead, and the cyst filled with caseous or calcareous matter. When, too, the fluid in the cyst is turbid, instead of clear and limpid, it is probable that the parasite is no longer living. Beef containing hydatids is only dangerous in the raw or imperfectly cooked condition.

'Measly' beef is very prevalent in India, but is not common in this country.

Tænia solium.—*General Characteristics.* — This tapeworm is smaller and contains fewer segments than *T. saginata*. In an extended state it is from 3 to 3·5 metres in length, but when contracted, as seen in preserved specimens, its length is usually less than 2 metres Its greatest breadth is about 8 mm. It usually has about 850 segments, of which from 80 to 100 are 'ripe.' It has a spherical head, about the size of a pin's head, provided with four prominent suckers, and from twenty-six to twenty-eight hooks. The apex is often marked with traces of a black pigment (fig. 31). As in the case of *T. saginata*, each proglottis, after leaving the parent colony, is capable of acting more or less like an independent organism (figs. 25 and 26).

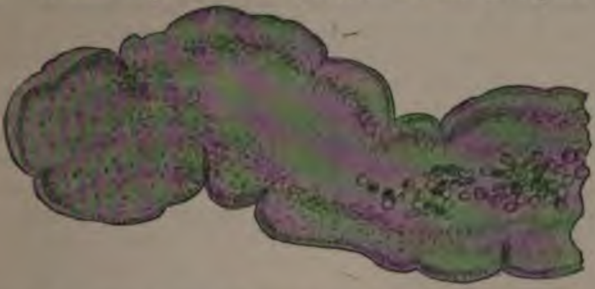

FIG. 26.—Portion of section of Proglottis of Tapeworm (*Tænia solium*). × 42. (After *R. M. Prideaux.*)

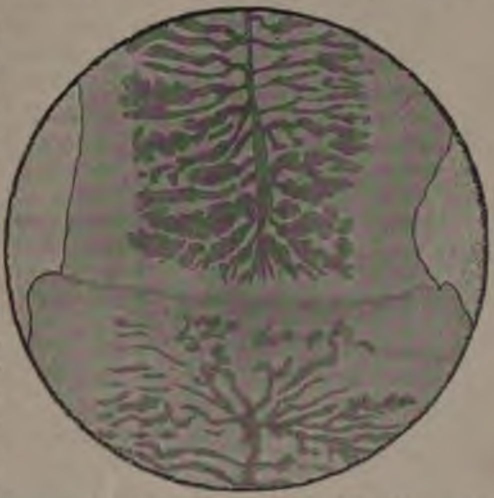

FIG. 27.—Section of ripe Proglottis of *T. solium*, with Sexual Organs. × 8. (After *R. M. Prideaux.*)

The term 'common tapeworm' is used collectively to indicate both this and the preceding tapeworm. According to Leuckart, Jews are free from *T. solium* (of the pig scolex), but are as much infected with *T. saginata* as the rest of the community. The uterus of a free proglottis is a characteristic structure (figs. 27 and 31).

The larva of this tapeworm (*C. cellulosæ*) is found most frequently, although by no means exclusively (*cf.* Table, p. 231) in the muscles of the pig, where it produces the well-known 'measles' of pork. Its origin and development take place in a similar manner to that of the ox-hydatid, but it is much more dangerous from the fact that the bladder-worm and tapeworm are capable of living in the same host, and thus, in the case of man, auto-infection with the hydatids, which produce much greater organic disturbances than the tapeworm, is by no means impossible.

'Measles' in Pork.—When eaten by a pig, the covering of the eggs of the tapeworm is dissolved by the gastric juice, and the embryo, piercing the wall of the intestine, chooses a suitable place in the muscle, and is gradually transformed into a hydatid, on the inner wall of which the head is developed. After three weeks the bladder is the size of a pin's head, and continues growing until the ninth week, when it is as large as a pea, and the head, on which the suckers and hooks can now be distinguished, is as large as a pin's head. After three months the neck is developed, and the larva is ready for transference to its subsequent host. It now becomes enveloped in a capsule of connective tissue, and remains quiescent until the animal dies or is killed.

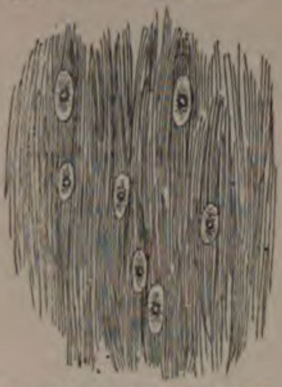
FIG. 28.—'Measles' in Pork. About two-thirds of the natural size. (After *E. Mitchell.*)

Unlike the *trichinæ*, which imbed themselves in the muscular fibre, the hydatids prefer the connective tissue between the fibres. Among the parts most frequently infected are the paunch, heart, tongue, neck, diaphragm, and inner side of the thigh. They are least frequently found in the liver, lungs, and intestine.

The frequent occurrence of the hydatids in the tongue often enables the owner of a pig to discover them in the living animal, and in Germany such infected swine are often promptly sent off to some out-of-the-way place where there is no inspection of meat, although the practice is forbidden by law.*

The cysts formed by the swine bladder-worms are somewhat smaller and rounder than those of the ox hydatids, and have not so grey an appearance. Calcareous degeneration also occurs with much less frequency. The living hydatid contains a clear transparent fluid in which the white head of the parasite is seen. The walls of the bladder are composed of a semi-transparent membrane, and around this the connective tissue of the flesh in which the worm is imbedded becomes thickened, forming an additional layer, so that the cyst has a greyish opalescent appearance (figs. 32 and 33). The head, retracted within the bladder, is furnished with four suckers, and a proboscis on which is a double circlet of from twenty-four to thirty hooks (figs. 29 and 31).

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* Fischoeder, *loc. cit.*, p. 164.

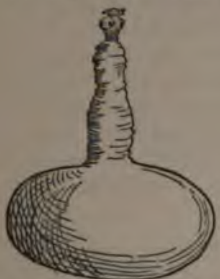

FIG. 29.—Swine Cysticercus with head protruded. × 3. (After *Leuckart.*)

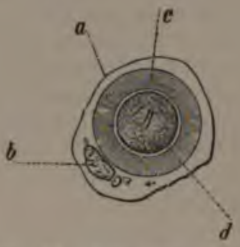

FIG. 30.—Ripe egg of *Tænia solium.* *a*, albuminous envelope; *b*, remains of yelk; *c*, covering of the embryo; *d*, embryo with embryonal hooklets. (*Landois and Stirling.*)

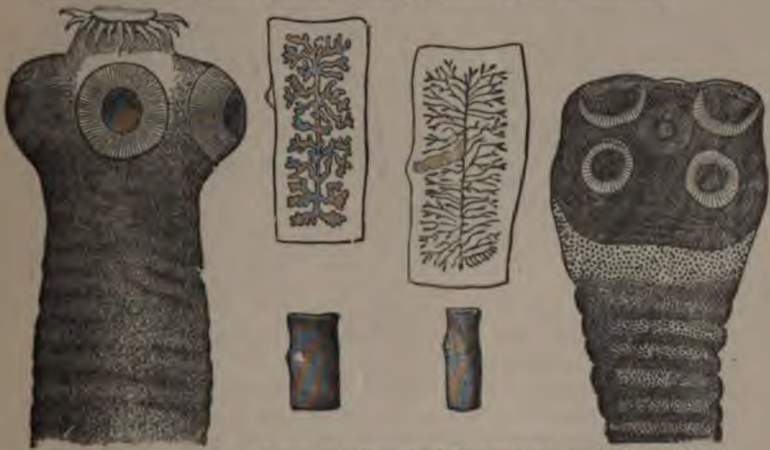

FIG. 31.—Head, etc., of *Tænia solium* and *T. saginata*, and joints of both, those above showing sexual organs. (*Landois and Stirling.*)

According to Fischoeder,[*] swine are infected relatively seldom (0·3 per cent.) in countries in which there is a regulated system of sewage disposal. As a rule only young pigs less than six months old become 'hosts,' although hydatids are sometimes found in animals more than eighteen months old.

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[*] *Loc. cit.*, p. 162.

Tænia acanthotrias.—Only the cysticercus of this tapeworm is known. In form it is very similar to *C. cellulosæ*, and, like it, is met with in the muscles and brain of man. It is distinguished by the form of its hooked organ of attachment, which consists of a triple circlet of from fourteen to twenty-six rather slender hooks. The related tapeworm is unknown, but probably lives in the human intestine. The cysticercus has hitherto only been met with in the human subject, but its occurrence in beef is also probable.*

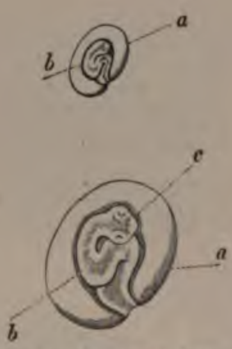

FIG. 32.—Cysticerci from *T. solium*. Natural size and magnified. *a*, embryo sac; *b*, cavity produced by budding of the embryo sac; *c*, suctorial discs and hooklets. (*Landois and Stirling.*)

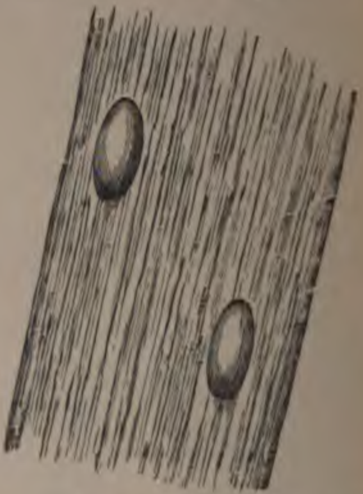

FIG. 33.—Encapsuled cysticerci from *T. solium*, imbedded in a human sartorius. Natural size. (*Landois and Stirling.*)

Tænia marginata.—*General Characteristics.*—This is one of the most common parasites of the dog. It is distinguished from *T. solium* by the form of its hooks, which are more slender, although of about the same size, and average from thirty-six to thirty-eight in number. Its suckers also are smaller and weaker. It sometimes attains a length of 2·5 metres, but, as a rule, does not exceed 1·5 metres. It has never been found in the human subject.

Cysticercus tenuicollis.—The related bladder-worm of *T. marginata* is found in the liver and viscera of ruminants and swine, and occasionally in deer, but its presence in man has never been proved beyond doubt. It has a strong resemblance in appearance to the bladder-worm of the hare (*C. pisiformis*). It has been found in a very young lamb's liver, forming pale yellow points visible to the naked eye. At an early stage of their existence the bladder-worms wander through the liver of the animal, forming long passages.

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* Leuckart, *loc. cit.*, p. 561.

As a general rule the bladder-worms do not remain in the liver until the end of their growth. Most of them pass into the body-cavity before the head (which in this species is formed at a late stage) has developed, and after remaining there for some time in a free state form fresh capsules for themselves, which sometimes grow to a great size. Leuckart* mentions that in the museum at Giessen there is one from an ox which is 160 cm. long and 6 to 7 cm. broad, and it is on record that a *tenuicollis* cyst measuring 12 inches by 4 has been found in a pig.

When flesh containing a bladder-worm is eaten by a dog, all but the head and neck of the parasite is destroyed, and in from ten to twelve weeks the resulting tapeworm has produced ripe *proglottides.*

Tænia serrata.—This tapeworm is also found in the dog, but does not pass into man. It has some resemblance in appearance to *T. solium*, for which it has occasionally been mistaken. It is distinguished from *T. marginata* by its larger head (1·3 mm.), which is provided with conspicuous suckers, a rostellum (0·64 mm.), and a double circle of from thirty-eight to forty-eight larger and more powerful hooks (fig. 34).

Cysticercus pisiformis.—This is the corresponding bladder-worm of this tapeworm, and is usually found in the liver of rabbits and hares. On the fourth or fifth day after eating the eggs the liver of the animals will be found studded with small white points resembling tubercles. These gradually increase in size until the third or fourth week, when, like the *Cysticercus tenuicollis*, the worms leave the liver for the body-cavity, and eventually become encysted in a fresh position. In the second week the young bladder-worms measure 0·5 mm. or more, and soon after change their globular form for a more extended one. About the third week they are about 2 mm. in length and 0·4 mm. broad, and begin to develop the characteristic head (fig. 34).

As in the case of the *coccidia*, the cysts within the liver are enveloped in a layer of connective tissue. The effects of this hydatid are not so severe as those caused by the *C. tenuicollis* in the liver of the sheep, for the latter, at the time of its exit, is considerably larger. Leuckart states that he has never met with a fatal case in rabbits, and that after the worms have left the liver the passages close up, leaving eventually only scars. In addition to rabbits and hares, any grass-feeding ruminant may become infected with this hydatid, though cases are not common.

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* *Loc. cit.*, p. 577.

Tænia cœnurus.—This is the smallest of the three similar tapeworms of the dog. It differs from *T. marginata* and *T. serrata* in the shape and structure of its head, and in the structure of the sexual organs. The head (0·8 mm. in diameter) is small and pear-shaped, and has from 24 to 32 hooks. In the complete tapeworm there are less than 200 joints between the head and the first ripe *proglottis*, whereas in the case of *T. marginata* and *T. serrata* the numbers are about 550 and 325 respectively. The full-grown worms vary in size from 20 to 50 inches. In England not more than 5 per cent. of the dogs become infected with it, but in Iceland it is very prevalent.* It has not been found in the human subject.

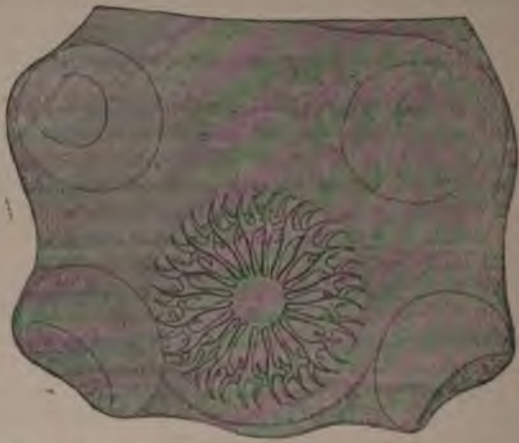

FIG. 34.—Head of *Cysticercus pisiformis*. × 30. (After *R. M. Prideaux.*)

Cœnurus cerebralis.—The larva of *T. cœnurus* is most commonly met with in the brain cavity of sheep, but it has also been found in the spinal marrow and subcutaneous tissue of sheep, and in the viscera of a squirrel and lemur.† It is a compound parasite. In most normal cysticerci only one head arises from the inner wall of the bladder, but the *cœnurus* can produce an unlimited number, and Eichler has found as many as 2000 in a single individual.[*] The heads, which, at an early stage, only number three or four, rapidly develop in colonies, as it were. Fig. 35 shows a section of the wall of the bladder of a *cœnurus* with some of the heads. Each head is capable of producing a sexually mature tapeworm in the dog in about three weeks. Apart from this characteristic formation of unlimited heads, the bladder-worms are similar in structure to other cystic bladder-worms. They form passages in the brain similar to those made in the liver by *C. pisiformis*, and produce what is known as 'gid' or 'staggers' in the sheep. The older an animal gets the more immune does it become against the attack of this bladder-worm, and, as a rule, only lambs are infected. With the advance of the disease the flesh of the animal greatly deteriorates.

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* Cobbold, *Internal Parasites of Domestic Animals*, p. 96.

† Cobbold, *loc. cit.*, p. 72.

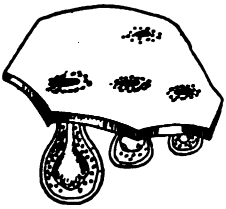

FIG. 35.—*Cœnurus cerebralis.* × 25. (After *Leuckart.*)

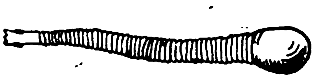

FIG. 36.—*Cysticercus fasciolaris* from mouse. ¾ natural size. (*Leuckart.*)

Tænia crassicollis.—This is a small tapeworm which inhabits the intestines of the common and wild cat.

Cysticercus fasciolaris.—This is the corresponding bladder-worm, and is found in rats and mice, its favourite position being the liver. It is one of the smallest cysticerci, the cyst rarely exceeding the size of a pea, and is notable from the fact that its head and body rapidly become too large for the bladder, and are protruded at an early stage of its existence. Owing to its jointed form it was once regarded as a complete *tænia*, but it has long been proved that the segmented body is destroyed like that of any other bladder-worm when the parasite is taken into its final host, and only the head is left to develop into the adult tapeworm (fig. 36).

Tænia tenella.—On several occasions Cobbold[†] met with, in the human subject, a slender tapeworm which he believed to be the adult form of the bladder-worm of the sheep (*Cysticercus ovis*), although feeding experiments gave negative results. It differed from *T. solium* in having shorter proglottides, and in the structure of the sexual organs.

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[*] Cobbold, *loc. cit.*, p. 98.

[†] *Entozoa of Man and Animals*, p. 98.

Cysticercus ovis.—It has often been asserted that no special bladder-worms are developed in the muscles of sheep, but Cobbold states that on five separate occasions he has detected a characteristic bladder-worm in mutton. The 'measles' were somewhat smaller than the similar cysticerci in pork, and the bladder-worms were quite distinct from the *Cysticerci bovis* and *cellulosæ.* The head was about $\frac{1}{30}$-inch in diameter, and was provided with four suckers $\frac{1}{100}$-inch across, and, unlike that of the bladder-worm of the ox, with a double crown of twenty-six hooks. The neck and head were studded with calcareous corpuscles, which were apparently very distinct on this cysticercus. He was unable to prove experimentally to what tapeworm this bladder-worm belonged. Leuckart points out that from Cobbold's description of this cysticercus it has a strong resemblance to the *C. cellulosæ* of the pig; but, on the other hand, all Leuckart's attempts to infect sheep with the eggs of *T. solium* were unsuccessful.*

GROUP B.

Tænia echinococcus.—In the group of the tæniadæ of which this tapeworm is representative, the heads of the larvæ are budded off from brood-capsules on the inner surface of the bladder. This tapeworm, which inhabits the intestines of the dog, wolf, and jackal, is very small, not exceeding 5 mm. in length, and 0·3 mm. in breadth. It has only three or four segments, of which the last is larger than the rest combined. The head is provided with strong, well-defined hooks, usually from twenty to thirty in number.

Echinococcus polymorphus or *Hydatid Bladder-Worm.*—The larva of this small tapeworm has long been known by the name of '*echinococcus*' or '*hydatid.*' It develops in the organs of all herbivorous animals, especially in the lungs and liver, forming conspicuous bladders, which often increase to an immense size by budding internally and externally. Unlike the cysticerci it is comparatively rarely found in muscle.

It is commonly classified into three varieties:—1. A simple bladder. 2. A bladder containing daughter bladders. 3. Composite bladders united by means of connective tissue. The last variety is usually met with in the ox and in man; the two first in ruminants (other than oxen), in swine, and in monkeys. In all three varieties the heads may be present or absent. As a general rule they are developed when the bladder is as large as a nut, but not infrequently at a later period. Thus Leuckart records an instance in which the lungs and liver of a cow contained 150 echinococci as large as a hen's egg, all of which were barren, whereas in other bladders, only 10 mm. in diameter, the formation

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* *Loc. cit.*, p. 498.

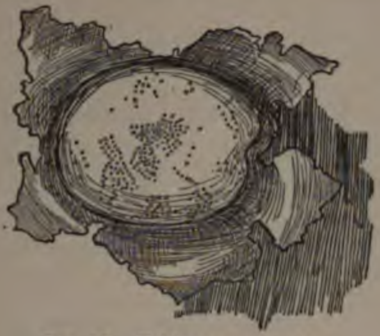

FIG. 37.—Echinococcus bladder. (*E. Mitchell*, after *Leuckart.*)

of heads had already commenced. Each head is capable of subsequently developing into a complete tapeworm, so that one bladder may produce a colony of worms in the dog.

In one or other of its forms the echinococcus is a frequent parasite in cattle, and causes considerable mortality. In the ox the bladder sometimes reaches a size of 12 inches or more in diameter. The hydatid in cattle is only indirectly dangerous to man, since the tapeworm does not develop in the human intestine. But he is readily infected with the eggs of the *tænia*, and the resulting echinococci often produce terrible effects. Cobbold states that in certain countries, notably Australia and Iceland, the disease is both prevalent and fatal.

Lücke[*] has examined the chemical nature of the echinococcus bladder, and finds that the substance is of a chitinous nature, but differs from the chitin of arthropoda in being less resistant to the action of boiling water and of potassium hydroxide. He states that there is a marked difference between the amount of ash in young and in old bladders (*e.g.*, 15·79 and 0·28 per cent.

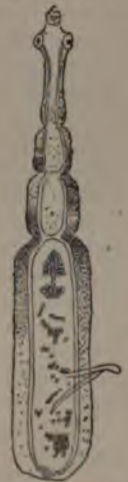

FIG. 38.—Tænia echinococcus. × 12. (*E. Mitchell*, after *Leuckart.*)

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[*] Leuckart, *loc. cit.*, p. 630.

respectively). He gives the following figures of the elementary percentage composition of the two (omitting ash):—

	C.	H.	N.	O.
Old Bladder, . . .	45·342	6·544	5·1493	42·9547
Young ,, . . .	44·068	6·707	4·478	44·747

II.—ORDINARY TAPEWORMS (CYSTOIDEI).

The tæniadæ in this division do not form bladder-like cysts in the larval stage, and the body of the 'bladder-worm' or *cysticercoid* is solid, or nearly solid. With the exception of cysticerci found in birds, such as *Piestocystis variabilis* in the crow, which appear to be intermediate, since their bodies often contain a little fluid, the cysticercoids are found only in cold-blooded animals, such as fish, worms, snails, and insects.

Tænia nana.—This is a very small tapeworm, 12 to 20 mm. in length. It has a spherical head with four round suckers, and twenty-two to twenty-eight very small hooks. It has hitherto only been found in the human subject in Egypt. The cysticercoid probably inhabits some snail or insect.

Tænia flavopunctata.—This is a tapeworm about 12 inches in length, which is characterised by having a central yellow spot on each unripe joint. Its head is club-shaped, and is devoid of rostellum or hooks.

Tænia madagascariensis.—A tapeworm about 8 cm. in length, only found once in the human subject in Madagascar.

Tænia cucumerina.—This is the tænia most frequently occurring in dogs or cats, as many as 200 individuals being sometimes met with in one animal. It is from 18 to 25 cm. in length, and its head has a club-shaped projection and four rows of about sixty hooks. It occurs as frequently in the cat as in the dog, and is identical with the varieties *T. canina*, *T. elliptica*, and *T. moniliformis*. It is not rare in man. The cysticercoid inhabits the dog-louse (*Trichodectes canis*).

Other Tæniadæ.—Only a passing allusion can be made here to the many other species of tapeworms which have been described, such as, for instance, *T. perfoliata* of the horse, *T. crassiceps* of the fox from *Cysticercus longicollis* in the shrew, and *T. tenuicollis* in the pole-cat from *C. talpæ*. The tæniæ found in birds are, as a rule, characterised by a well-developed rostellum as in *T. paradoxa* in the oyster-catcher. Other examples of avian tapeworms are *T. malleus* in the domestic fowl, *T. microps* in the capercailzie and grouse, and *T. infundibuliformis* in the grouse, partridge, and quail. The cysticercoids from which these are developed probably live in different kinds of insects, or in snails.

Family II.—The Bothriocephalidæ.—The parasites belonging to this family of the cestoda are of simpler construction than the tæniadæ. The tapeworms attach themselves to their hosts by means of two longitudinal suctorial grooves, but do not possess true suckers or a rostellum, and the head, which is flat and oval, is as a rule devoid of hooks. The segments are less defined than in the more common cestoda, and the openings of the reproductive organs of the individual joints are differently situated. In most cases the larva at some stage of its existence lives in an intermediate host, but never seems to become a true bladder-worm, although Leuckart states that in some instances the body has an appendage which apparently corresponds to the bladders of the cysticerci of beef and pork. The larvæ develop in the muscle, or more commonly in the viscera or liver of animals associated with water (frogs, or other reptiles, and birds), but especially in fresh-water fishes.

Bothriocephalus latus (*The Broad or Pit-Headed Tapeworm*).—This is the best known representative of this family. It is common in parts of Russia, Switzerland, Sweden, and N.-E. Germany, and though rare in England, is said to be indigenous in Ireland.* It has hence been termed the Irish tapeworm. When full grown it sometimes attains a length of 25 feet, and may have as many as 3000 to 3500 short joints. The body is thin, flat, and ribbon-like, and, unlike the *tæniadæ*, the joints are not separated off as separate organisms, but are expelled in connected chains.

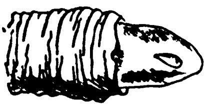

FIG. 39.—Head of *B. latus* reared in a cat from the larva from a pike. × 21. (After *Braun.*)

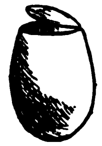

FIG. 40.—Ovum of *B. latus.* × 280. (After *Leuckart.*)

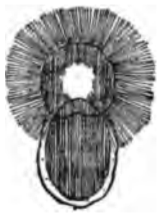

FIG. 41.—Ciliated embryo of *B. latus* emerging from the ovum. × 200. (After *Leuckart.*)

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[*] Cobbold, *Tapeworms*, p. 17.

The head is long, oval, and pointed, and has two longitudinal grooves, which serve as suckers (fig. 39).

The eggs are oval and comparatively large (0·05 mm. in diameter), and have a characteristic operculum. When immersed in water they develop into ciliated embryos, which give birth, as it were, to a higher form of larva (pro-scolex), which has a diameter of 0·045 mm., and is provided with six well-developed hooks, with which it makes its way into the tissue. This develops into a still higher larva (scolex), which becomes encysted in the pike or other river fish, but there is no knowledge as to how the change occurs. Leuckart and others have kept young pike in water with the ciliated embryos without infection taking place, and the former suggests that there may be two intermediate hosts, the embryo being eaten by some small aquatic invertebrate animal, which in turn is eaten by the fish.

The Higher Larva of B. latus.—As found encapsuled in the muscle or on the intestinal wall this larva is an inconspicuous, flat, club-shaped organism from 5 to 10 mm. in length and 2 to 3 mm. in breadth (figs. 42 and 43).

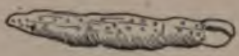

FIG. 42.—Higher larva of *B. latus*, with head retracted, from pike. × 6. (*Leuckart.*)

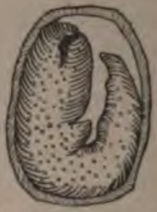

FIG. 43. — Higher larva of *B. latus* encapsuled. × 13. (*E. Mitchell*, after Braun.)

The head, which is usually retracted, has suction grooves similar to those of the adult worm. The body is solid, and is studded with numerous calcareous particles, so that the larva has a white appearance. It is not segmented, and, unlike the flesh cysticerci, has no bladder. The capsule in which it envelops itself is soft, and the head often projects outside it, especially in the case of those on the intestinal walls. When removed from the capsule, and placed in warm and moist conditions, the larva moves actively, bending and straightening itself. Leuckart * states that, under favourable conditions, it is capable of existing for eight days outside its host. It has been proved that this higher larva develops into *B. latus* in man and in the cat, but curiously enough the tapeworm in the latter is distinguished by having very many less joints, though otherwise identical. The method of infection appears to be through eating imperfectly cooked fish. Braun found that smoked pike was dangerous, and also that the larva could remain alive in the fish when frozen hard. Although the pike is the most common intermediate host, the larva also occurs in the turbot, and probably in members of the salmon and trout family. As the tapeworm is very prevalent among the Jews in Utrecht, who are great bleak-eaters,* it is not improbable that in its intermediate stage it should also inhabit that fish. Recently† von Schröder has found the larva in the perch. Eighty fishes from Dorpat were examined, and of these 35 per cent. were infected, though only to a slight extent.

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* *Loc. cit.*, p. 718.

Bothriocephalus cordatus.—This is not unlike the preceding tapeworm as regards the structure of its joints, but is much smaller (about 12 inches long), and has a small, broad, heart-shaped head. It is frequently found in the dog in Greenland, where it also occurs in the seal, walrus, and man. In this country it is most likely to be met with among the inhabitants of the islands on the northern and western coasts.* The higher larva has not been identified, but probably lives in marine fishes.

Bothriocephalus cristatus.—This is a rare tapeworm of from 8 to 10 feet in length, which is characterised by a crest-like rostellum. It has twice been found in France. Its higher larva is not known.

Bothriocephalus liguloides. — This has hitherto only been observed in China and Japan.

Malformations of the Cestoda.

It is by no means uncommon for abnormal forms of the cestoda to be met with both in the complete tapeworms and, what is of more importance in the examination of flesh, in the larval stage. Thus, among the tæniadæ, double monsters have been described, and formation of supernumerary joints is common, especially in the beef tapeworm (*T. saginata*). The most common malformation in *Bothriocephalus latus* is the presence of double genital apertures in the joints.

The occurrence of monstrosities with more than four suckers and with an unusual number of hooks is not infrequent, both in the tapeworm and its bladder-worm. Thus Klepp ‡ describes one which was the only individual (*Cysticercus cellulosæ*), found in a pig. This had six suckers instead of four, and twenty-eight hooks.

Referring to this case Zürn § gives a summary of such abnormalities in various bladder-worms as recorded by different observers. Küchenmeister, he states, describes bladder-worms of

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* Cobbold, *Entozoa*, p. 111.

† *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 55.

‡ *Ibid.*, 1898, p. 207.

§ *Ibid.*, p. 228.

T. solium and *T. saginata* with six suckers. Raillet (*Notices parasitol.*) has observed the deformity in the hare cysticercus (*C. pisiformis*), and it has frequently been noticed in the case of *Cysticercus tenuicollis*. According to Leuckart and Küchenmeister the abnormality is not rare in *Cœnurus cerebrulis*, and Bremser has met with a *Tænia crassicollis* with six suckers. Bladder-worms with five suckers have also been described (Gomez).

The Temperatures at which Cysticerci perish.

It is a matter of great importance to know what is the highest temperature that different bladder-worms can resist, so as to determine what degree of cooking is necessary to render them harmless when encysted in meat. It was formerly believed, from inconclusive experiments, that the bladder-worms of beef and pork did not perish at 100° C., but Professor Perroncito of Turin, in a systematic series of experiments, the results of which he communicated to Dr. Cobbold, proved that the temperature was much lower. The method which he employed was to immerse the free cysticerci in water, or a dilute solution of sodium chloride, and to raise the temperature gradually. As the water became warmer they continued moving their suckers and probosces, until when a certain temperature was reached they suddenly became motionless. As it was then possible to stain the parasites with an aniline colour, there could be no question as to their being dead. The time taken to heat the water was about ten minutes in each experiment. The temperature was raised from 8°–10° C. to 45°–46° C. in six to eight minutes, and from 46° to 50° C. in about one minute. The following is a summary of Perroncito's principal results and conclusions:—

1. *Cysticercus cellulosæ* dies sometimes at 45° C., more frequently at 47° C., and ordinarily at 49° C. In exceptional cases it can resist a temperature of 50° C. for a few moments. Hence, if the cysticercus be gradually heated to 50° C., and maintained at that temperature for a minute, it undoubtedly perishes.

2. *Cysticercus bovis* perishes between 44° and 45° C., and in any case is unable to withstand a temperature above 46° C.

3. *Cysticercus pisiformis* of the rabbit sometimes perishes between 45° and 46° C., but usually becomes motionless at that temperature, and dies at 47° to 48° C.

4. *Cysticercus tenuicollis* dies at 49° C.

5. The scolices of *Cœnurus cerebralis* die at 42° C.

7. The scolices of the cysts of *Echinococcus polymorphus* usually perish between 47° and 48°, and never resist a temperature of 50° C.

A number of similar experiments were also made with different species of complete tapeworms. *Tænia cucumerina* dies at 43° to 45 C.; *T. perfoliata* of the horse at 45° to 50° C., and *T. serrata* at 50° C.

From these results Perroncito concluded that 'measly' meat is quite harmless when it has been cooked so that the temperature throughout is maintained at 50° C. for five minutes. His conclusions were confirmed in a very practical manner by some of his pupils, who voluntarily ate some infected meat before and after being heated to that temperature, with positive results in the first instance and negative results in the second.

Leuckart points out that in dishes which are rapidly cooked, such as sausages and cutlets, the temperature required to destroy the cysticerci may not be reached in the interior of the flesh, and that this would account for some of the cases of tapeworms in persons who have never eaten raw meat.

According to Cobbold the ova of tapeworms do not lose their vitality when exposed to the action of frost. From the experiments of Braun on the larva of the *B. latus* (p. 247), and from the observations of others on the cysticerci in beef and pork, there appears to be little doubt but that bladder-worms can withstand a considerable degree of cold.

Recent experiments, however, have shown that prolonged refrigeration is fatal to cysticerci in flesh. After being kept for twenty-one days in a chamber in which a constantly low temperature was maintained the cysticerci were readily dissolved in artificial digestion experiments, which would not have been the case with living cysticerci, and no tapeworms were produced when animals were fed with the meat which contained them.

By a recent Prussian regulation (November 1897), the flesh of oxen and calves which are only slightly infected with cysticerci (*i.e.*, does not contain more than ten living parasites) is allowed to be sold under police regulation, provided it has been either—(1) thoroughly cooked, or (2) kept for twenty-one days in a pickle containing 25 per cent. of salt, or (3) kept for twenty-one days in a suitable refrigerating-chamber, in which the temperature is from 3° to (at most) 7° C., and in which the amount of moisture in the air has not exceeded from 70 to 75 per cent.

Influence of Putrefaction on Cysticerci.

Cobbold* states that cysticerci have been found alive in all parts of a leg of pork which had not become putrid twenty-nine days after the pig had been killed. In a similar experiment on veal the cysticerci were all dead in fourteen days. According to Fischoeder† they lose all power of development when flesh is left for three weeks, even without salting or pickling. Leuckart[*] also found that bladder-worms perished during putrefaction of the flesh containing them. In midsummer they became flabby and turbid in eight days, although in autumn and winter they were alive and in motion after being kept for fourteen days at a temperature of 5° to 8° C.

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* *Entozoa*, p. 70.

† *Leitfaden der Fleischbeschau*, p. 167.

Cysticerci in Ham.—From Leuckart's experiments[*] it appears that ham prepared from 'measly' pork is quite harmless. During the smoking the fluid in the bladder of the scolex disappears, the body becomes turbid, and collapses, and even in fresh ham cysticerci were never found alive. Küchenmeister obtained similar results in his experiments. Ostertag[†] states that they also perish when the meat is kept in brine for twenty-four hours.

The Examination of Flesh for Cysticerci.

The beef and pork cysticerci and the larva of the *Bothriocephalus latus* can often be detected by the naked eye in sections of the flesh, but in sausages and other meat preparations the aid of the microscope is necessary.

Kissling[‡] treats the selected portions of the sausage with a dilute solution of potassium or sodium hydroxide (sp. gr. 1·15), so as to isolate the cysticerci.

Schmidt Mulheim advocates the use of acid pepsin for the same purpose. The flesh is treated with a solution of 100 c.c. of 0·5 per cent. hydrochloric acid and 5 c.c. of glycerin pepsin. When cysticerci are present the surrounding tissue is dissolved, and the heads of the parasites sink to the bottom of the liquid, where they appear like minute grains of rice.

In order to determine whether the cysticerci which may have been found without chemical treatment of the flesh are alive or dead, the condition of the liquid in the bladder should be noted (*vide supra*).

As a further test they may be isolated and examined microscopically by Perroncito's method (*cf.* p. 248).

The parasite is placed in water in a cell on the slide, the temperature gradually raised to 50° C., and an observation made whether any movements occur during the warming.

The staining test is also a valuable criterion. The living bladder-worm, and more especially its head, cannot be permanently stained with hæmatoxylin or carmine, whereas the tissue when dead readily retains the stain.

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[*] *Die menschl. Parasiten*, p. 534.

[†] *Handbuch der Fleischbeschau*, p. 269.

[‡] *Zeit. Fleisch u. Milch Hyg.*, 1896, vi. Heft. 4.

THE TREMATODA, OR LIVER FLUKES.

The parasites commonly known as 'liver flukes' have considerable external resemblance to the isolated proglottides of tapeworms. They are flat, leaf-shaped organisms, provided with suctorial apparatus, and sometimes with hooks of attachment. The only members of this group which need be described here are two belonging to the *distomata*. These have two suckers, a mouth, and a digestive apparatus, and the embryos are developed in an intermediate host.

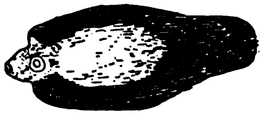

FIG. 44.—Common Liver Fluke. Natural size. (*Leuckart.*)

Distomum hepaticum.—This is the common liver fluke, which was first described by Gabucinus in 1547. When sexually mature it is a large, hermaphrodite parasite, sometimes as much as an inch in length. Its body is long, flat, and oval, and covered with cuticle studded with rows of spines. The eggs are large and oval, and develop in water into globular embryos, which undergo a further metamorphosis in the body of the fresh-water mollusc (*Limnæus*), where they become the higher larvæ of the fluke. These higher larvæ, or *cercaria*, are colourless, and have a tail appendage, by means of which they can swim about in the water. After a time they encapsule themselves on an aquatic plant, or a stick or a stone, and wait until they are swallowed by a higher animal, when they penetrate into the liver or other part of the body and become complete liver flukes.

They are most common in the sheep and ox, but have been found in many other herbivorous animals, including the elephant, pig, hare, rabbit, and kangaroo. In man they are also met with occasionally, the cercaria having probably been swallowed with water-cress or some other plant. On several occasions they have become almost epidemic, causing great mortality. For example, Leuckart states that, in 1873, 33 per cent. of the total sheep in Alsace-Lorraine were destroyed by them; and in 1882 not less than a million sheep are said to have perished in the southern province of Buenos Ayres from their attack.

Distomum lanceolatum.—This is the only other liver fluke of common occurrence in domestic animals in this country. It is much smaller than the last parasite, being only 8 to 9 mm. in length. It has a thin, lancet-shaped body, with pointed ends, and differs considerably in its internal construction. It undergoes a metamorphosis similar to that of the common liver fluke, and is often found associated with it in the liver.

Occasionally individuals of both kinds of fluke make their way into the lungs, where they form a hard cyst containing a dark-brown, turbid liquid, within which the parasite can be distinguished. They may also become encysted in other organs, such as the spleen, or even in the muscles.

In the stage of development in which they occur in flesh they are not injurious to man.

Other Liver Flukes.—A passing reference may be made here to *D. Rathouisi*, which resembles *D. hepaticum*, and has been found in the human subject in China; to *D. pulmonale*, a thick, plump parasite found in the lungs of animals and men in China, Japan, and Corea; to *D. crassum*, the large human fluke; and to *Fasciola gigantea* of the giraffe.

THE TRICHINA SPIRALIS.*—This parasite, which belongs to the Nematoda, or round worms, is the only member of the order which need be described at length in the present work. It was probably first discovered in 1822, when Tiedemann noticed certain capsules in muscles which he described as 'concretions.' In 1833 Hilton came to the conclusion that these 'concretions' were of a parasitic nature, a view which was confirmed in the following year by Paget, who found the worm in the cyst. In 1835 Owen named and described it, but it was not until 1860 that Zenker proved the connection between the trichina and the terrible disease now known as *trichinosis*. Finally, its life history has since then been fully investigated and described by Virchow and by Leuckart.†

Life History of the Trichina.—When an animal eats flesh containing living, encysted trichinæ, the calcium carbonate of the capsule is dissolved by the gastric juice, and the young worms, set free, rapidly grow into adult worms in the intestines, where in the course of a few days the females produce young, and then die. These young trichinæ (*embryos*) penetrate into the muscles of their host in enormous numbers, and, having chosen a suitable spot, each coils itself up and becomes surrounded with a cyst, which in the course of a few months becomes calcified. Here it remains quiescent until the flesh is eaten, when, like its parent, it develops into the sexually mature parasite.

A distinction must thus be made between the intestinal trichinæ and muscle trichinæ, the latter being the immature stage of the former.

Intestinal Trichinæ.—These minute worms have been found or induced to live in the intestines of man, the pig, wild-boar, dog, rat, mouse, calf, lamb, foal, rabbit, guinea-pig, birds, and other animals. The body is round and thread-like, pointed in front and blunt behind. The mouth opens into a canal leading to the œsophagus, which is surrounded by a series of connected bladder-like cells containing granular protoplasm. These extend more than half the length of the body, and are known as the 'cell-structure.' Küchenmeister and Zürn[*] consider that this characteristic structure probably fulfils the function of glands. The œsophagus is continued into a stomach cavity, which opens into an intestinal canal. The organs of generation are distinct in the two sexes.

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* From the Greek θριξ (=hair).

† Cobbold, *Human Parasites*, p. 31.

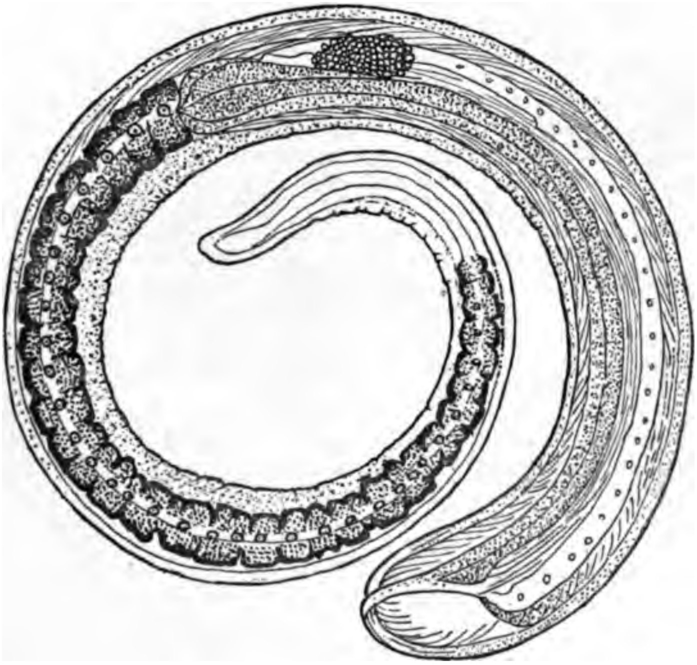

FIG. 45.—Immature Female Trichina. × 360. (After *E. Mitchell.*)

The full-grown male measures at most 1½ mm. in length, and is provided with two external hooked claws towards the posterior part of the body. The female is considerably larger, averaging from 3½ to 4 mm. in length. The muscle trichinæ develop into intestinal trichinæ in from thirty to forty hours, and in six or seven days the females, which considerably exceed the males in number, begin to produce young, and continue to do so until the sixth or eighth week, when they die. The male trichinæ die at a much earlier period.

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[*] *Die thierischen Parasiten im Menschen*, p. 453.

Embryo Trichinæ.—The young trichinæ or embryos are brought forth alive, each female producing some 1500 or more. They are only about 0·01 mm. in length at the time of their birth, but rapidly develop, and when about 0·12 to 0·16 mm. long, pierce the walls of the intestine, pass into the body cavity, and thence into the muscles. A later view * is that the female deposits the young not in the intestinal cavity, but in the walls of the intestine itself, whence they pass with the lymph secretion into the blood, and so are directly introduced into the muscles. The greatest number of wandering trichinæ are met with in nine to twelve days after the infection of the host.

[illegible] *Trichinæ.*—After the young trichinæ have penetrated into the selected muscular fibres they remain quiet for about fourteen or sixteen days in order to complete their growth. When about [illegible] in length they coil themselves into a spiral or S [illegible] The space in which they lie becomes a wide, [illegible] within which a cyst is gradually formed, [illegible] or ninth week and being completed [illegible] week.

[illegible] The muscle trichina measures [illegible] mm. in breadth. [illegible] narrower than the back. The [illegible] although the sexual organs [illegible] distinguish between the [illegible]

[illegible] The muscle trichinæ can develop [illegible] intestinal trichinæ [illegible] whose intestines [illegible] have never been [illegible] in the pig [illegible] in which the [illegible]

[illegible] attacked with [illegible] muscles are [illegible] and thick. [illegible] structure. [illegible]

[illegible]

in length by 0·25 mm. in breadth. It has a sharply outlined double border, and contains a liquid in which are fine round granules, and within which the coiled worm can be discerned. When the animal in which the trichina is encysted is in good condition fat cells are sometimes deposited at the extremity of the cyst. As a general rule only one individual is found within a cyst, but in cases of strong infection the cyst may contain as many as six trichinæ.

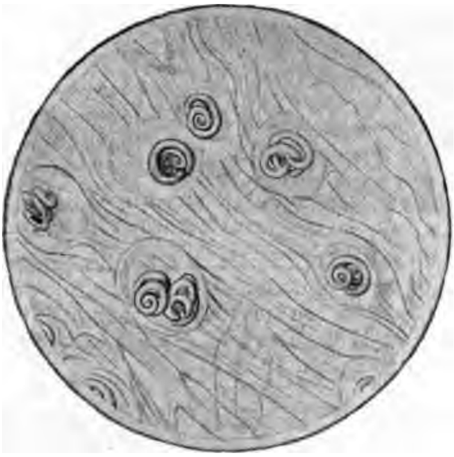

Fig. 46.—Encysted Trichinæ in Human Flesh. × 33. (After *Prideaux.*)

After about six months the cysts become calcified, at first towards the ends, and the whole cyst may be enveloped in a calcareous deposit in the course of sixteen or eighteen months. It then appears dark throughout, and the trichina is only faintly visible or is quite invisible until the calcium carbonate of the cyst is dissolved with acid (figs. 47 and 48).

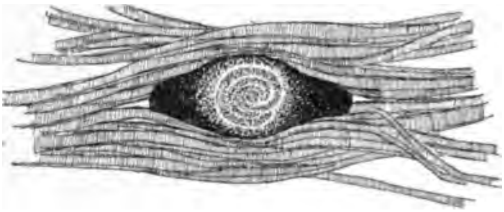

Fig. 47.—Calcified Muscle Trichina with Parasite faintly visible. × about 60. (After *Long* and *Preusse.*)

Muscle trichinæ can live encysted in flesh for many years. For example, they have been found alive and capable of development $11\frac{1}{2}$ years after their first invasion of the muscle, and Tungel* computed that some he discovered alive in human muscle must have been encysted for $13\frac{1}{2}$ years.

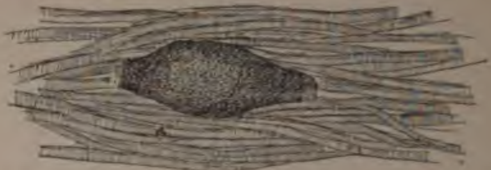

FIG. 48.—Calcified Muscle Trichina, with Parasite invisible. × about 60. (After *Long* and *Preusse*.)

At the same time it is probable that the trichina usually dies at an earlier stage, its body undergoing fatty or calcareous degeneration, and the calcification may be so complete that when the calcium carbonate is dissolved with dilute acid nothing remains of the original worm (figs. 49 and 50).

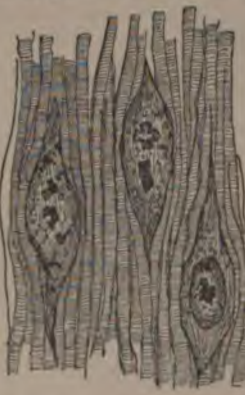

FIG. 49.—Dead calcified Trichinæ. (After *Leuckart*.)

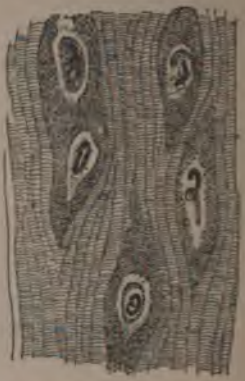

FIG. 50.—Dead calcified and disintegrated Trichinæ. (After *Leuckart*.)

Effect of the Trichinæ on the Host.—In the pig, which is the animal most frequently attacked by the muscle trichinæ, the symptoms of the disease are often not very severe, and the animal may show no signs of the infection. Only in exceptional cases do the worms develop so rapidly that death ensues. In human beings, however, the results are generally much more serious, the wandering trichinæ causing sharp pains in the muscles, flaccidity of the limbs, fever, peritonitis, paralysis, etc., and, when many of them have been eaten, death may rapidly ensue. In some cases, however, the acute symptoms cease when the trichinæ have become encysted, and the patient may then regain his normal health.

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* Küchenmeister and Zürn, *loc. cit.*, p. 456.

Number of Trichinæ in Infected Flesh.

Cobbold states * that in an outbreak of trichinosis in Cumberland in 1871, the pork which produced the epidemic contained upwards of 80,000 trichinæ in one ounce, and from this he calculates that one pound of such flesh would be capable of producing some 400,000,000 flesh worms in the subsequent human bearer. Leuckart calculated that the flesh of an infected cat contained 325,000 trichinæ, and in another case found 1500 individuals in one gramme of flesh. Cobbold also computed the number in the total muscles of a man who had died of trichinosis at from 90 to 100 millions.

The Detection of Trichinæ in Flesh.

Method of Sampling.—In taking samples from isolated pieces of meat such as ham, pieces about the size of a hazel-nut are cut out in the longitudinal direction of the muscle fibres, and as near as possible to where the muscle is attached to sinews or bones.

Where the whole animal is being examined, special attention must also be paid to the muscles in which the trichinæ most frequently occur (p. 254).

In examining sausages Fischoeder † recommends the cutting of four thin slices from each kilogramme and picking out with a needle, for microscopical examination, all particles which from their paler appearance and finer fibres appear to consist of pig's flesh.

In the case of American hams the test sections should be taken from the deep-lying muscles. Trichinæ are not found in the fat.

Microscopical Examination.—For the microscopical examination thin sections of the samples are made parallel with the fibres. These are placed on the object glass, with a cover glass pressed down on them, and examined under an amplification of twenty or thirty times.

The Compressor.—In order to make them completely transparent, and to examine a number of preparations rapidly, Fischoeder advocates the use of a *compressor* (fig. 51). This consists of two glass plates from 12 to 15 cm. in length and about 3 to 5 cm. broad, which fit accurately upon one another, and are connected by means of two delicate adjustable screws at the ends. The compressor is divided into twenty-four equal fields by means of twelve transverse lines on the lower plate, and a broad opaque band on the upper plate. This apparatus is not applicable with the higher powers of the microscope, but serves its purpose very well with the low powers. The various thin sections are placed in the numbered divisions of the compressor and the screws gradually tightened until the flesh becomes quite transparent.

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* *Entozoa*, p. 156.

† *Loc. cit.*, p. 224.

Treatment of Opaque Sections.—In the examination of fresh juicy flesh, clear preparations are readily obtained without any other treatment than pressure. But in other cases a special treatment with a reagent is necessary. Thus, to dried or smoked flesh is added either a drop of an 0·75 per cent. solution of sodium chloride, or of 3 per cent. acetic acid, or of 30 per cent. potassium hydroxide mixed with three parts of water. Glycerin is also sometimes useful in rendering the preparation transparent. When a substance resembling the trichina cyst in appearance is found, it should be carefully examined with higher powers, after treatment with acetic acid to dissolve the lime, in order to determine whether a trichina or parts of a trichina are contained within it.

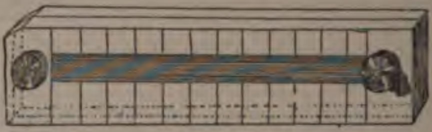
FIG. 51.—Fischoeder's Compressor.

Treatment of the Flesh with Pepsin.—Schimdt-Mulheim's method of isolating cysticerci (page 250) by treating the flesh with an acid solution of pepsin is also applicable to the detection of encapsuled trichinæ, the cysts falling to the bottom of the vessel.

Examination with the Röntgen Rays.—Recent experiments carried out at the University of Würtzburg have shown that calcified trichinæ can be detected by means of the Röntgen rays. But since non-calcified trichinæ, which are of much more frequent occurrence in pork, are not capable of detection in this way, the method is useless for the practical examination of meat.

Parasites and other bodies which might be mistaken for Trichinæ.—No other round worms which might be mistaken for trichinæ are met with in pigs' flesh. But other bodies are frequently found which at first sight might be mistaken for the cysts formed by the trichinæ.

1. *Miescher's Tubes.*—These parasites were described on page 228. They differ from trichinæ cysts in their shape and in the fact that the muscle fibres surrounding them have not lost their

vertical and horizontal striations. Moreover the substance contained in the Miescher's tubes is regularly distributed and the calcification commences from the interior outwards instead of from the ends as in the trichinæ cysts. When completely calcified, Miescher's tubes can usually be no longer recognised, whilst the trichinæ, after treating the capsules with dilute acid, can often be identified.*

2. *The Muscle Ray-Fungus* (*'Strahlenpilz'*).—This is occasionally found in the muscular fibres of the pig and in those of the sheep and calf. It is a defined dark body with a lighter centre and with a radiating structure (fig. 52). The surrounding muscle fibres have a dirty brown colour, and in places lose their cross striations, or in severe cases become altogether disintegrated. At a later period calcium carbonate is deposited. The favourite muscles of this fungus are those of the diaphragm, paunch, and side. It can be distinguished from the trichinæ cysts by its rounder form, by its radiating structure (which in exceptional cases can be made out after dissolving the calcium carbonate with dilute acid) and by its size. *Cf.* p. 296.

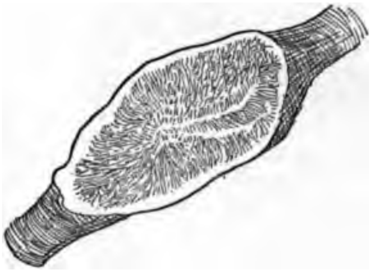

FIG. 52.—Muscle Ray-fungus. × 160. (After *Ostertag.*)

Calcium Carbonate Deposits.—Calcium carbonate is sometimes deposited in the muscle of the pig and (less frequently) of the sheep. Sometimes the concretions are so small as to be invisible to the naked eye, while in other cases they reach an appreciable size (fig. 53). They may occur in any of the muscles, and may be either isolated or in such quantities that the flesh has a greyish appearance. They are most frequently met with in the muscles of the diaphragm, paunch, and inside of the ham. According to Fischoeder † they invariably originate from Miescher's tubes in the case of sheep, whereas in swine they may have been caused by various parasites, such as the ray-fungus, cysticerci, trichinæ, echinococci, or Miescher's tubes.

Ostertag ‡ gives the following notes for distinguishing between the calcareous deposits caused by different parasites:—

(*a*) The muscle ray-fungi lie in the muscular fibres, do not form capsules, and are arranged like a string of pearls. They are sometimes found in the cardiac muscles.

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* Fischoeder, *loc. cit.*, p. 230.

† *Loc. cit.*, p. 232.

‡ *Handbuch der Fleischbeschau.*

(*b*) Miescher's tubes lie *in* the muscle fibres, which do not lose their cross striation, and have a soft cuticle which is dissolved by potassium hydroxide solution. Calcification proceeds from the interior.

(*c*) Trichinæ lie *in* the muscles. If the parasite died before the calcification, no signs of it may be found on dissolving the calcium carbonate with acid, and only the facts that the formation is about 1 cm. long and spindle-shaped, and that the surrounding muscular fibres are altered, point to its having originated from a trichina. If, on the other hand, the calcification took place after the formation of the cyst, the latter may be readily identified after treatment with dilute acid.

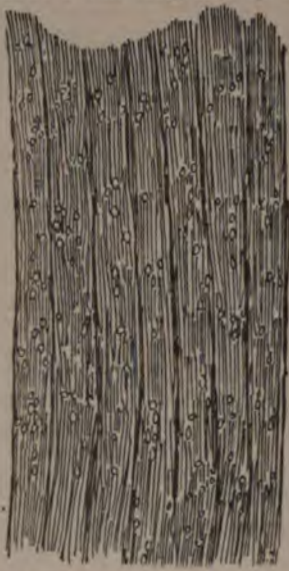

FIG. 53.—Calcareous deposit in muscle. ×2. (*Heller.*)

(*d*) Cysticerci are invariably larger, lie *between* the fibres, and have an exterior envelope of connective tissue, and in the older parasites the hook and calcium deposits in the parasite can be observed.

(*e*) Echinococci are very rarely met with in muscle, and only when the internal organs are strongly affected. They lie *between* the muscular fibres and vary greatly in size. They are distinguished from the *cysticerci* by their so-called '*echinococcus skin*' with its characteristic markings (p. 242).

(*f*) The deposition of crystals which are occasionally found in smoked pork or ham is not due to the presence of parasites but to purely chemical alteration. Under the microscope they appear as irregular masses which extend over several muscular fibres (fig. 54). They can be distinguished from the calcareous deposits originating from parasites, by the fact that they are soluble in dilute solutions of potassium hydroxide but not in dilute acid.

Tyrosine Deposits.—Occasionally a deposition of tyrosine has been observed in ham. Schmidt-Culmbach* records a recent instance of this in which the flesh contained numerous small accretions from 1 to 3 mm. in size, deposited on the fibres. They were found to consist of tyrosine.

* *Zeit. Fleisch u. Milch. Hyg.*, 1898, 8, p. 150.

Muscle Distomum.—This is a parasite very rarely met with in pork. It is of extremely soft consistency and of a greyish colour and is about the same size as a trichina cyst, from which however it is distinguished by lying between the muscular fibres. Moreover, on warming it moves vigorously. Under a microscope its internal structure can be made out. According to Fischoeder * the presence of this organism is without significance in flesh.

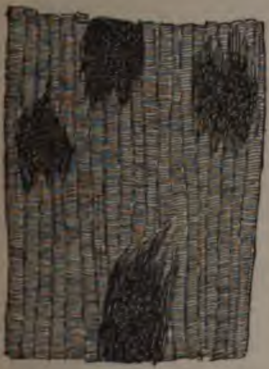

FIG. 54.—Crystalline deposit in smoked ham. (*Leuckart.*)

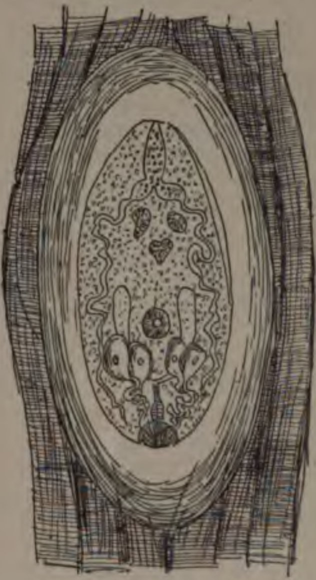

FIG. 55.—Muscle distomum. (*Leuckart.*)

The Temperatures at which Trichinæ Perish.—Perroncito in the course of his experiments on cysticerci (p. 248) made a similar research on the power of trichinæ to resist heat, and found that in every case the parasite, whether in the free state or encysted, perished when exposed to a temperature of 48° C. for five minutes. On the other hand it appears to be able to withstand a low temperature, for, at least, some considerable time. Thus Küchenmeister and Zürn state that trichinæ have been found alive after the flesh had been kept frozen for over two months, † and Leuckart found that they were little injured after exposure to a temperature of − 25° C. for three days.

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* *Loc. cit.*, p. 233.

† *Die thierischen Parasiten im Menschen*, p. 472.

The Destruction of Trichinæ in Flesh.—(*a*) *Cooking.*—From Perroncito's experiments it appears that in order to render infected flesh harmless it should be cooked, so that a temperature of not less than 50° C. is maintained throughout every portion of the meat for not less than five minutes.

Küchenmeister[*] made a number of experiments to determine what is the temperature ordinarily reached throughout the whole piece during the cooking of various kinds of meat in the ordinary way. He obtained the following results:—Broiled flesh, 60° C.; roast sausage, 62·5° C.; boiled beef, 87·5° C.; beefsteak (2), grilled, 56·3° C. and 57·5° C.; cutlet, 62·5° C.; roast pork (middle of joint), 65° C.; liver sausage, 90° C., and blood sausage, 90° C.

Perroncito examined still more thoroughly the temperatures reached during cooking,[†] and of the large number of experiments which he carried out the following may be given as typical:—

i. A piece of veal (7 cm. by 25 cm.) had a temperature of 53° C. in the centre after being boiled for ten minutes. After being boiled for twenty minutes the temperatures in different central parts were 63°, 65°, and 66° C.

ii. Beef (8 cm. by 10 cm.) placed in boiling water had after twenty minutes, a blood-red centre in which the temperature was 47° C. After thirty-five minutes the temperature in the interior was 68° to 70° C.

iii. A ham of about 6 kilos. in weight was placed in cold water and boiled. When the water boiled the temperature in the interior of the ham was 25° C. After thirty-five minutes it was 35° to 40° C., and after two hours the temperature in different parts of the interior were 46°, 55°, 58°, 62°, 64°, and 67° C.

iv. A ham of about 8 kilos. treated in the same way showed an interior temperature of 44·5° C. after two and a half hours. After three and a half hours the temperatures in different central parts were 62°, 65°, 74°, 78·5°, and 84° C. Hence it is evident that when an ordinary-sized ham is cooked for three to three and a half hours, a temperature is reached in every part which is more than sufficient to destroy any trichinæ.

The danger of insufficiently-cooked flesh is shown by Kühn's experiments, in which pigs were fed with partially-cooked pork containing trichinæ. A piece of about 4 pounds was boiled for one hour and thirty-nine minutes, and the pig to which it had been given was killed some time afterwards. Only very few trichinæ were found in its muscle. In another experiment 4½ pounds of the infected flesh were boiled for two hours and fifteen minutes and given to a young pig, in which subsequently only one trichina was found in 270 test samples taken from its flesh.

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[*] *Loc. cit.*, p. 467.

[†] Küchenmeister, *loc. cit.*, p. 468.

In the outbreak in Cumberland (p. 257) Cobbold[*] states that the source of infection was a home-fed pig, the flesh of which had been made up into sausages, which were eaten when only imperfectly cooked. Three members of the family were attacked with trichinæ, but all subsequently recovered.

(*b*) *Salting.*—Attacks of trichinosis after eating salted meat are very rare. König[†] suggests that there is probably a process of double decomposition between the salt and the calcium carbonate of the cyst, with the formation of calcium chloride and sodium carbonate which dissolve, so that the parasite is exposed and perishes.

Küchenmeister[‡] states that strips of infected pork, after being salted and placed in a vessel of water for a short time, contained no living trichinæ.

Leuckart[§] asserts that if infected flesh is rubbed with salt and left without water for four weeks, dry salt being rubbed in at intervals during the pickling, it will contain no living trichinæ at the end of that time.

On the other hand, Gerlach[‡] found living trichinæ in imperfectly salted flesh after eight weeks.

In Eckart's quick-salting process (*cf.* p. 107) any trichinæ that may be present are completely destroyed.

Lehmann[||] states that in the ordinary method of salting, trichinæ perish in the upper layers (2 to 3 cm. deep) after some weeks, but that in the interior of the ham they have been found alive two months after salting.

(*c*) *Smoking.*—Cold smoking of flesh, or the so-called 'quick smoking,' is ineffectual as a means of destroying trichinæ. But according to Küchenmeister,[¶] hot smoking, if properly carried out, destroys all parasites. Thus, in the case of ham, no trichinæ were alive after ten days' smoking in a hot chamber, and the same result was attained with sausages after twenty-four hours' hot smoking. Lehmann[**] states that in well-smoked hams, such as American hams, they are usually dead.

Influence of Putrefaction on Trichinæ.—Trichinæ appear to have much more power of resisting the effects of putrefaction than the cysticerci have, and have been found alive after 100 days in decomposing flesh.

Occurrence of Trichinæ and of Trichinosis.—During the first six years after the trichinæ were known as the cause of trichinosis

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[*] *Entozoa*, p. 169.

[†] *Nahr. Genussmitt.*, ii. p. 96.

[‡] *Loc. cit.*, p. 470.

[§] *Die menschlichen Parasiten*, 1st ed., p. 598.

[||] *Methods of Practical Hygiene.*

[¶] *Loc. cit.*, p. 471.

[**] *Loc. cit.*

(1860–1865), no less than twenty-six epidemics of the disease were recorded in different parts of Germany. Muscle trichinæ appear to be fairly common parasites in pork, though owing to the inspection of flesh in some countries, and to the usually thorough cooking in others, outbreaks of trichinosis do not now occur very frequently. According to Küchenmeister,* from 5 to 8 per cent. of the pigs slaughtered in America are infected with trichinæ, and yet trichinosis is very rare in that country. In Germany, the prevalent custom of eating raw ham must be held accountable for many of the outbreaks of the disease, and the microscopical examination of the flesh by inspectors, however thoroughly carried out, affords no absolute certainty that the meat is free from infection.

C. Bockelmann † calls attention to the large number of infected sausages imported into Germany from America, and the necessity of having them more thoroughly inspected. He mentions several cases that have come under his personal notice in Aachen. One, for example, in which a consignment of sixty cases of sausages was received, in which eleven cases contained sausages in which were trichinæ. In consequence of this and similar instances, the sale of American sausages in Aachen has completely ceased.

Edelmann ‡ states that the pigs' livers imported into Dresden from America or Denmark frequently contain trichinæ. In the year 1896 four livers out of 2023 were found to be infected. In each case, however, the parasites were dead, probably owing to the action of the antiseptics used to preserve the flesh.

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* *Loc. cit.*, p. 472.

† *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 64.

‡ *Ibid.*, p. 34.

CHAPTER XIII.

THE BACTERIOLOGICAL EXAMINATION OF FLESH.

By means of a bacteriological examination it is often possible to supplement the information to be obtained from a chemical and microscopical examination of flesh. Thus, in certain cases in which the meat is discoloured, the morphological and biological characters of the micro-organisms present may enable one to determine the cause of the discoloration. Similarly the determination of the number of organisms in preserved meat or sausages may enable one to form a judgment as to the wholesomeness of a preparation, as, for example, when the characteristic bacteria of putrefaction (*B. proteus vulgaris*, etc.) are found in large numbers.

On all fresh meat numerous organisms will be found, some of which produce innocuous products, while others are harmless so long as they have been unable to decompose the flesh to a sufficient extent to form those poisonous products which, when absorbed into an animal's system, cause *sapræmia*, or '*putrid intoxication*.'

The Species of Bacteria on Flesh.—The various forms of bacteria may be classified into three groups—*bacilli*, *micrococci*, and *spirilla*, the different varieties of which are shown in fig. 56. Representatives of all these may be found in sound or diseased flesh. For the description of the nature, properties, and various systems of classifying bacteria, and for general methods of sterilising, preparation of culture media, etc., the reader is referred to one of the general treatises on bacteriology.

Bacteriological Methods of Examining Flesh.—*Preparation of Tissue Sections.*—Small pieces of the flesh are hardened by immersion in alcohol for three or four days, or in Müller's fluid (potassium bichromate, 2 parts; sodium sulphate, 1 part; water, 100 parts) for a fortnight or more. When hardened the alcoholic preparations are soaked in water; those hardened in Müller's solution simply dried on blotting paper.

Before cutting the sections it is necessary to embed the tissues. Paraffin or celloidin are suitable substances for embedding.

Embedding in Paraffin.—The hardened tissue is dehydrated by being steeped in alcohol for two to six hours. After the alcohol has been removed by means of turpentine, benzene, or xylol, the tissue is immersed in melted paraffin (*m. p.* about 50° C.) just before it begins to set. When cold the sections are cut, the paraffin removed by means of turpentine or xylol, the xylol displaced by alcohol, and the section washed with water and stained.

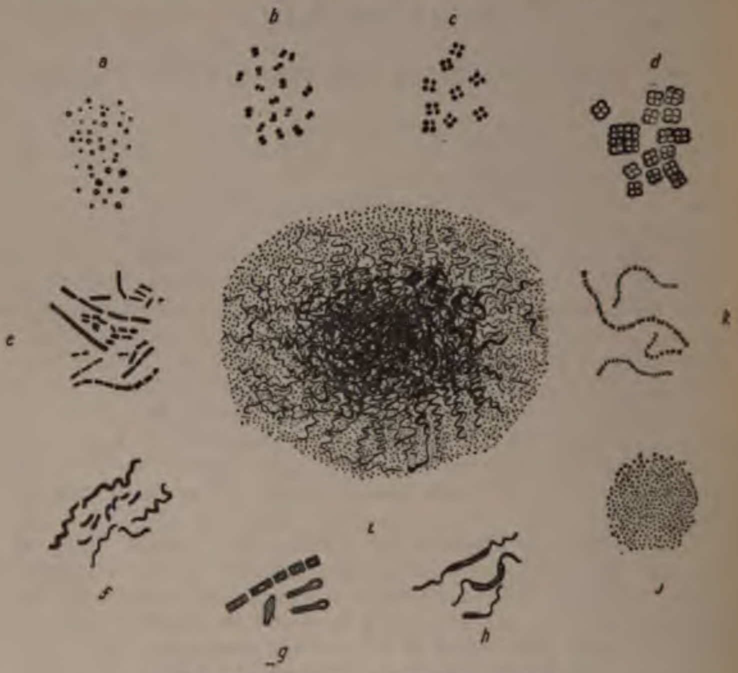

FIG. 56.—Bacteria. (After *Baumgarten.*)

a, Cocci; *b*, Diplococci; *c*, Tetracocci; *d*, Sarcinæ; *e*, Bacilli; *f*, Spirilla; *g*, Bacteria with spores; *h*, Bacteria with flagella; *i*, Zoogleæ; *j*, Staphylococci; *k*, Streptococci.

Embedding in Celloidin (Nitro-cellulose).—The hardened tissue is left for one or two days in a mixture of equal volumes of alcohol and ether. It is then steeped in a dilute solution (in ether and alcohol) of celloidin, and then transferred for some time to a solution of about the consistency of a syrup. It is next placed in a small porcelain crucible and covered with the celloidin solution. The crucible is immersed in 80 per cent. alcohol for twenty-four hours, after which the embedded tissue is cut out, placed in water till it sinks, then transferred to gum, frozen, and cut with a microtome.

Section Cutting.—A simple and convenient apparatus for cutting a small number of sections is shown in fig. 57, which represents Ranvier's Hand Microtome.

In this the embedded tissue is gradually raised by means of the screw beneath, and the successive sections cut with a razor.

Swift's Freezing Microtome (fig. 58) is an instrument in general use. Ether is used as the freezing agent with it.

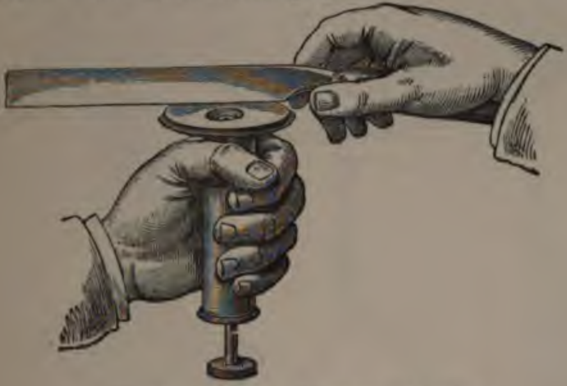

FIG. 57.—Ranvier's Microtome. (*Stirling.*)

Methods of Staining Tissues.—The thin sections obtained as described above may be stained by a number of methods, of which two of the best known are those of Gram and of Weigert.

Gram's Method in Outline.—(1) Aniline gentian violet, 10 minutes. (2) Iodine solution (I, one part; KI, 2 parts; water, 100 parts), 30 seconds to 1 minute. (3) Alcohol until decolorised. (4) Eosin solution for 2 minutes. (5) Rinse in alcohol. (6) Oil of cloves for 2 minutes. (7) Mount in Canada balsam after removing the oil with blotting paper.

Weigert's Method.—(1) Immerse the sections for 6 to 18 hours in a 1 per cent. aqueous solution of a basic aniline colour (methyl violet, gentian violet, fuchsin, Bismarck brown). (2) Wash with water. (3) Pass through 60 per cent. alcohol into absolute alcohol until *nearly* decolorised. (4) Stain with Weigert's picro-carmine solution for 30 minutes. (5) Water. (6) Alcohol. (7) Clove oil. (8) Dry on filter paper and mount in Canada balsam.

Determination of the Number and Species of Micro-organisms in Flesh.—A weighed fragment of the flesh is thoroughly mixed with 50 c.c. of sterilised water, or nutrient fluid, and plate cultivations made with definite quantities of the liquid. The number of colonies obtained are counted by one of the ordinary bacteria counters.

In order to determine the nature of the bacteria the different species are isolated by sub-cultivations of the colonies. To cultivate anaërobic bacteria the cultivations are made in the depth of grape sugar, gelatin or broth, which are inoculated below the surface by means of a capillary pipette, the test tube containing them being subsequently sealed with paraffin.

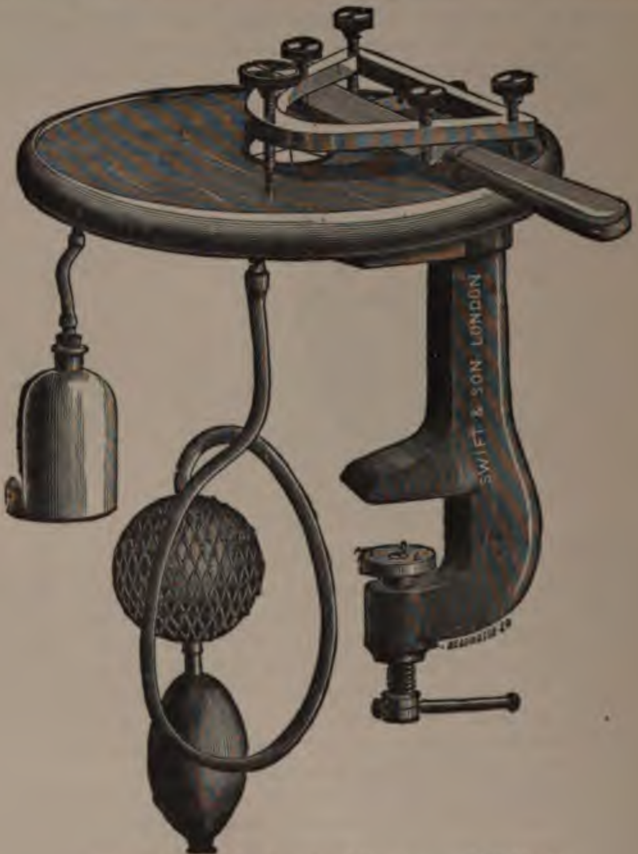

FIG. 58.—Swift's Freezing Microtome. (*Stirling.*)

Description of the more important Bacteria of Flesh.

The Bacteria of Normal Flesh.—Many of the numerous species of micro-organisms which have been isolated from fresh and preserved meat and fish are identical with the bacteria often met with in putrefying meat. Kraus [*] made a number of investigations as to the nature of the bacteria present in raw beef, veal, and pork, not less than twenty-four hours after the death of the animal. He found that the number of species present, as well as the number of bacteria, increased with the age of the flesh. In one case he found fifty-one kinds on the outside of the meat. Five species were constantly met with. (1) A bacillus resembling *B. coli communis*; (2) a similar species in smaller numbers; (3) a species which, on cultivation, formed a superficial growth with characteristic wrinkling; (4) a bacillus resembling *B. subtilis*, which, cultivated on potatoes, formed a reddish-yellow granular layer; (5) a bacterium forming a brownish-yellow growth. From the results of his experiments Kraus came to the following conclusions:—1. Different kinds of flesh do not have special species of bacteria. 2. The number of species varies with the time of year. 3. In those cases in which the injection of juice from putrefying flesh was fatal to mice, the same bacillus was found in the organs of the mouse as in the flesh juice. 4. This bacillus appears to be identical with Gärtner's *B. enteritidis*, and is not pathogenic in fresh flesh, though it becomes pathogenic in the presence of saprophytes.

According to Gärtner, meat which is three days old only contains bacteria on the outside; after ten days, when putrefaction has set in, the bacteria are found to a depth of 1 cm., but the blood-vessels are free if the animal was healthy when killed.

Bacteria in Sausages and Smoked Flesh.—A. Serafini [†] made a bacteriological examination of a large number of sausages, and found in almost every case a bacillus which, from its appearance on cultivation, he concluded to be identical with Flügge's *B. mesentericus vulgatus* (*cf.* p. 275). In his opinion this was derived from the sausage skin, and not from the meat. In addition to this bacillus, others of various species were found, some of which were inert, and others so active that they completely decomposed the sausage in a few days.

Deetjen [‡] made quantitative determinations of the number of bacteria in Lyons sausage, and found the following numbers in 1 gramme of the meat before and after boiling:—

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[*] *Jahresber. Nahr. u. Genussm.*, 1891, p. 37.

[†] *Ibid.*, 1892, p. 44.

[‡] Dissertation, Würzburg, 1890.

Raw, fresh,	1,894,000
After four days, raw,	6,654,000
After boiling for fifteen minutes,	61,000
After boiling for thirty minutes,	9,000

Sausages obtained direct from the maker contained from 324,000 to 448,000 bacteria per gramme in summer, and from 14,000 to 37,000 in winter.

In old 'Cervelat' sausage the numbers found amounted to 5½ millions. Among the species found was a spore-bearing bacillus, with a strong resemblance to *B. mesentericus vulgatus.*

In twenty-seven different kinds of fresh sausages and other flesh products which Schattenmann* examined, fourteen were found to contain *B. proteus vulgaris.* It was also found alive together with large numbers of other bacteria in smoked sausage and smoked flesh. Schattenmann agrees with Beu and with Ungaro that smoked bacon is free from bacteria inside. In Hamburg smoked beef, in 'Cervelat' sausage and in ham (in the fat as well as the lean) he found several species, including *B. proteus vulgaris* on several occasions.

Among the bacteria from various kinds of smoked flesh, Beu † identified *B. proteus vulgaris*, *Staphylococcus cereus albus*, *Staphylococcus pyogenes aureus*, and *B. liquefaciens viridis.*

Chromogenic Bacteria of Flesh.

There are several well-known bacteria widely distributed in the air, which form different-coloured pigments as products of their development on a suitable medium. Some of them do not, as a rule, grow readily on flesh, probably owing to the antagonism of other bacteria, such as those which commonly bring about putrefaction. Occasionally, however, these may fall upon the meat in such numbers as to obtain the upper hand, and so impart the characteristic colour of their pigments to the meat. This is notably the case with the *Bacillus prodigiosus*, which is not of very common occurrence on meat.

The chromogenic bacteria of flesh may be grouped according to the colour of their pigments.

Bacteria producing Red Pigments.—*B. prodigiosus.*—Bordoni-Uffrazi ‡ records an instance in which the public health office in

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* *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 189.

† Sternberg, *Bacteriology*, p. 591.

‡ *Zeit. Nahr. Untersuch.*, 1894, p. 219.

Turin received a half-cooked hen which had assumed a brilliant red colour in the night. On examination it was found to be infected with this bacillus.

Klein * mentions a similar instance of infection by this bacillus. In this case the whole of the flesh in a city establishment (beef, mutton, and fish) became red. The larder which contained them overlooked a churchyard in which the graves had recently been disturbed.

B. prodigiosus is a short bacillus with rounded ends. It occasionally forms filaments, sometimes resembles a micrococcus in form, and is frequently met with in pairs or in chains of ten or more. It is aërobic and facultatively anaërobic, usually non-motile, and liquefies gelatin. The pigment, which is an excretory product, requires oxygen for its formation. It is soluble in alcohol and ether, but insoluble in water. Acids change the colour to pale red, which, on the addition of alkali, is reconverted into the original colour. The pink coloration is only observed *en masse* and not in an individual bacillus. The micro-organism grows best at a temperature of 20° C. It occurs in air, water and soil.

Bacillus of 'Red Cod' (Dantec).—Found on dried salt cod which had turned red and had an offensive odour. A bacillus with rounded ends, 4 to 12 μ long, usually with a terminal spore. It resembles the *B. tetani*, but is considerably thicker. Grown on dried cod it forms circular reddish colonies.

Micrococcus of Dantec.—Another micro-organism isolated from red salt cod by Dantec in 1891. It is 3 to 5 μ in diameter, and often shows a line of commencing division. It is aërobic and non-liquefying. Grown on gelatin plates it forms small red disc-shaped colonies which rarely exceed 1 mm. in diameter. On dried cod-fish it grows without the production of a red pigment, except when certain other bacteria, notably a micrococcus which causes liquefaction, are simultaneously present.

Bacillus on Sardines.—This was found by Auche on the sardines, to which it imparted a bright red colour (*cf.* p. 115).

Bacteria producing Blue, Violet, or Green Pigments.—*Bacillus fluorescens liquefaciens* (Flügge).—Very common in putrefying infusions of meat. Short bacilli occurring in pairs. Liquefying, motile, aërobic. Spore formation not observed. Grown on gelatin plates it produces a green fluorescent pigment. In stab cultivations it forms a white growth along the line of inoculations. Liquefaction takes place in the upper part of the tube and the gelatin assumes a greenish-yellow fluorescence. On potato it forms a thick brown growth.

B. pyocyaneus (Gessard, 1882).—One of the organisms found in putrefying animal matter. A very small thin bacillus, 1 μ long, 0·3 μ broad, with rounded ends, frequently in pairs or in chains of 4 or 6, and occasionally in filaments. Spore formation (Crookshank). Does not form spores (Sternberg). On gelatin plates forms white colonies, liquefies the gelatin and imparts to it a fluorescent green colour. The green pigment, which is only formed in the presence of oxygen, is soluble in chloroform, and can be obtained in blue needles from the solution. According to Gessard (1890) the bacillus produces two pigments—one fluorescent green, the other blue (pyocyanin). On potato it forms a reddish-brown growth, which becomes green with ammonia. It is pathogenic to guinea-pigs and rabbits. It perishes at a temperature of 56° C.

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* *Micro-organisms and Disease*, p. 200.

B. janthinus (Zopf).—A putrefactive bacillus. Small slender rods with rounded ends, 2 μ long and 0·5 to 0·6 μ broad. Sometimes forms filaments. It is motile, and liquefies gelatin. In stab cultivations grows without the product of pigment along the line of puncture, but forms a thin violet layer on the surface. On agar-agar it produces a layer of a dark violet colour. It grows in milk without causing coagulation, but the liquid turns violet. It reduces nitrates to nitrites very rapidly.

B. erythrosporus (Eidam).—Found in putrefying infusions of meat. A slender bacillus with rounded ends, which often develops in short filaments. It is aërobic, motile, and non-liquefying, and produces oval spores, each filament containing from 2 to 8. In cultivations it forms a greenish-yellow fluorescent pigment. The colonies formed on gelatin plates are white and wrinkled, and the surrounding gelatin fluoresces greenish-yellow. The growth on potato is reddish at first, but subsequently brown.

The Bacillus of Grey Flesh.—Cases have been observed in which flesh has assumed a grey coloration, usually after being made up into sausages. Falk and Oppermann * found that in the latter case it was caused by bacilli on the interior of the sausage skins, and could be prevented by previously treating these with potassium permanganate.

The Bacteria of Phosphorescent Flesh.

The phenomenon of the spontaneous emission of light by fish and meat has long been known, and many curious instances are met with in old literature. According to Lemery † it was of frequent occurrence in Padua in 1492, and in the early part of last century became almost epidemic in Orleans, many butchers having to destroy their meat wholesale, owing to their customers refusing to buy it. A similar outbreak of phosphorescence on meat in Orleans occurred in 1870. In 1676 a Dr. Beale, of Yeovil, in Staffordshire, recorded in the *Transactions of the Royal Society* an interesting case in which a neck of veal suddenly became phosphorescent "and shined so brightly that it did put the woman into great affrightment," and mentioned as a possible explanation of the phenomenon that the weather was very warm and mild and the stars very bright on that evening. The veal was eaten on the following day without any ill effects.

Isolated instances of meat of every kind and of sausages becoming luminous are continually being recorded, many of these originating from the meat having been kept in the same larder as herrings or other marine fish, on which phosphorescent bacteria are of very common occurrence.

In 1800 a Dr. Hulme made a number of experiments to determine the cause of the phenomenon, and came to the following conclusions:—(1) It is not due to putrefaction, since phosphorescent meat has no offensive smell, and the luminosity decreases with the advance of decomposition. (2) Spontaneous light is a constitutional principle incorporated with the whole substance of certain bodies, and is probably the first principle which escapes from the body after the death of marine fishes.

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* *Deutsch. Fleisch Zeit.*, 1892.

† Lemery, *Of Chemistry*, 1720.

In 1877 Nüsch detected bacteria on phosphorescent meat, and in 1879 C. Baucel and C. Husson showed that the light emitted by a phosphorescent lobster was due to bacterial action. Since then many species of bacteria causing phosphorescence have been discovered, some of them being widely distributed.

The temperature has a considerable influence on their powers of luminosity. Ludwig found that although phosphorescent meat remained luminous when cooled to – 14° C., it ceased to emit light when warmed to between 20° and 30° C. But there is a difference in the behaviour of different species of bacteria in this respect. Thus, for instance, *B. indicus* thrives at 30° C., but will not develop below 15° C.

The light emitted by cultivations of the different species varies in character and intensity. In some cases it is bluish-white, and in others greenish-yellow. Ludwig, who examined spectroscopically the light produced by one species, found the spectrum to be very rich in violet rays. Fischer, too, succeeded in photographing a colony of *B. phosphorescens indicus* by its own light, using a very sensitive plate, and giving it an exposure of twenty-four hours.

The presence of oxygen is essential for the production of luminosity in cultivations, for, although some of the bacteria can readily be grown anaërobically, the colonies do not emit light. Cultivations made in the dark are phosphorescent, so that sunlight does not appear to be an essential factor.

Lehmann and Tollhausen suggest that the production of light is a vital process of the bacteria produced by internal molecular change within the cell, and this view receives some support from the fact that all chemical agents (acids, alkalies, etc.) which destroy the protoplasm of the cell, simultaneously put an end to the phosphorescence. On the other hand it is not improbable that the phosphorescence originates from an excretory product of the bacteria. And it is interesting to note in this connection that Dubois * extracted from the mantle of the luminous mollusc *Pholas dactylus* two crystalline phosphorescent compounds, one of which he named *luciferase.*

The principal phosphorescent bacteria which have been isolated are:—

B. phosphorescens gelidus (Forster). — Short rods with slightly rounded ends, about three times as long as broad. In old cultivations more nearly oval. On gelatin plates form round greyish colonies with often a yellowish-green tint. Aërobic and non-liquefying. Grown on potato they form a broad white layer. The cultivations emit light between 0° and 20° C., the phosphorescence ceasing at 32° C.

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* *Comptes Rend.*, 1887.

B. argenteo-phosphorescens, No. 1 (Katz).—Thin bacilli with pointed ends, 2·5 μ long and 0·8 μ broad, occurring singly or in pairs, and occasionally in long filaments. Aërobic, motile, and non-liquefying. Spore formation has not been observed. Form pale yellow colonies, emitting a silvery-white light. The phosphorescence depends on the presence of salt in the cultivation and on the free access of oxygen.

B. argenteo-phosphorescens, No. 2 (Katz).—Isolated from fish in Sydney market. Bacilli with rounded ends, 2·7 μ long, 0·67 μ broad, occasionally in short filaments. Non-motile, non-liquefying, aërobic. Spore formation not observed. Form pale yellow colonies, emitting a silvery-white phosphorescence.

B. argenteo-phosphorescens, No. 3 (Katz).—Isolated from phosphorescent cuttlefish. Resemble No. 2, but the rods are thinner and are motile. Frequently occur in pairs, and sometimes in short filaments. Aërobic and non-liquefying. Form a viscous yellow growth on sterilised fish. The light emitted is 'bluish-greenish white.'

B. smaragdino-phosphorescens (Katz, 1891).—Bacilli with pointed ends, 2 μ long and 1 μ broad, occurring singly or in pairs, and in old cultivations assuming the form of cocci. Aërobic, non-liquefying, and non-motile. Spore formation not observed. On gelatin plates form greyish-white colonies which emit an emerald-green phosphorescene.

B. cyaneo-phosphorescens (Katz).—Bacilli with round ends, 2·6 μ long and 1 μ thick, occurring singly or in pairs, and occasionally in long filaments. Very closely related to the *B. phosphorescens* of Fischer. Aërobic and facultatively anaërobic, non-motile, and liquefying. Stained by Gram's method. The surface colonies on gelatin plates are pale yellowish-green. On sterilised fish the growth is glistening yellow or pale brown. There is no growth on potato.

B. argenteo-phosphorescens liquefaciens (Katz). — Straight or slightly curved rods with round ends, 2 μ long and 0·8 μ broad. Sometimes form filaments. Aërobic and facultatively anaërobic, non-motile, and liquefying. On gelatin plates form light brown colonies. Grow on sterilised fish as a shiny, viscid, yellowish, grey layer, emitting a silvery phosphorescence, not so intense as that of the preceding species. No growth on potato.

B. phosphorescens indicus (Fischer).—Bacilli with round or pointed ends, from two to three times as long as broad. Aërobic, motile, and liquefying. Produce greyish-white colonies on gelatin, and a thin, broad, white layer on potato. Found by Fischer in the water of the Gulf of Mexico.

B. phosphorescens indigenus (Fischer). — Bacilli 1·3 μ long and 0·4 to 0·7 μ broad, occurring singly, in pairs, and in filaments. On gelatin plates form circular, greenish colonies, becoming yellowish at a later stage. No growth on potato.

The Bacteria of Putrefaction in Meat.

The micro-organisms which are capable of breaking down the constituents of flesh into simpler substances are extremely numerous, and the species which may be met with in any given case of putrefaction will depend largely on the length of time which has elapsed between the death of the animal and the removal of the intestines, as well as on the development of species derived from external sources. After death, bacteria normally present in the intestines penetrate into the abdominal cavity, and eventually, by way of the blood-vessels, into all the tissues. At the commencement of decomposition several species of micrococci and large bacilli are usually met with, but these subsequently give place to short motile bacilli of a more aërobic character, which were formerly grouped under the title of 'bacterium termo.'

The following bacteria have been isolated from putrefying meat, and one of them (*B. proteus vulgaris*) is believed by Hauser to be the special organism of putrefaction.

B. enteritidis (Gärtner).—Isolated in 1888 from the flesh of a cow which had caused fatal illness. It was also found in the spleen of the dead patient. In form it is a short, thick, motile bacillus with rounded ends. Spores are not known to occur. On gelatin it produces a greyish film without liquefaction. Gärtner never found this organism in the flesh of freshly-killed animals. Experiments with sterilised broth cultivations proved that the toxine formed by the bacillus was very virulent.

B. mesentericus vulgatus (Flügge).—'The Potato bacillus.' This is very widely distributed in air, dust, and on the surface of the potato. It was found by Seraphini in decomposing sausages. It is a thick bacillus, 1·2 µ to 3·5 µ in length, often occurring in pairs or in chains of several individuals. It is aërobic, liquefies gelatin, and forms spherical spores. Grown on gelatin plates it produces at first bluish, almost transparent colonies, with subsequently opaque white centres. In stab cultivations liquefaction occurs along the line of inoculation, while a greyish-white wrinkled growth is formed on the surface. When grown in milk it coagulates the casein, which is afterwards dissolved, and floats as a thin layer on the surface.

B. mesentericus fuscus (Flügge).—A small, short bacillus, which occurs singly, in pairs, or in chains. It is motile, and forms spores. The colonies formed on gelatin are circular, and at first dirty white, subsequently becoming brownish-yellow. Liquefaction takes place rapidly.

Micrococcus fœtidus (Klamann).—Cocci 1·4 µ in diameter. They occur singly, in pairs, in chains, or in irregular groups. Grown on gelatin plates they form white colonies, and liquefy the gelatin. In stab cultivations they grow as a white shining mass with a central prominence, surrounded by concentric circles, liquefaction being slow, and a disagreeable odour produced. At a later stage the growth acquires a brown colour. On potato the growth has a greyish-red colour, and its surface is rough.

B. coprogenes fœtidus. — Discovered by Schottelius (1885). It is a non-motile bacillus with rounded ends. Forms spores which are oval and arranged in rows, when there is free access of air. In gelatin stab cultivations forms a grey layer on the surface and pale yellow colonies along the line of inoculation. When grown on potato forms a dry, greyish layer 0·5 m. thick. In small quantities is non-pathogenic to rabbits and mice.

B. saprogenes (Rosenbach, 1884). — I. Large rods, spore formation. On agar-agar forms an irregular streak with viscous appearance, and emits a characteristic smell. Non-pathogenic. II. Rods thinner and shorter than I. On agar-agar grow as transparent globules which afterwards become grey.

Cause putrefaction in the absence of oxygen, but less rapidly than in its presence. Pathogenic to rabbits in considerable quantities.

B. putrificus coli (Bienstock). — A thin bacillus, about 3 μ in length, sometimes shorter. Frequently forms filaments. It is actively motile, forms a large terminal, spherical spore, and progresses with the spore in front. Grown on gelatin it produces a thin layer with an iridescent lustre, which later assumes a yellow colour. It is constantly present in fæces, and according to Bienstock is one of the chief factors in the decomposition of albuminous matter.

B. pyogenes fœtidus (Passet, 1885). — A short motile rod, 1·45 μ long and 0·58 μ broad. Usually occurs in pairs or in short chains. Spore formation (?) in the interior. Grown on gelatin plates produces white dots after twenty-four hours, which subsequently coalesce into a grey layer. The gelatin is not liquefied. In stab cultivations forms on the surface a greyish-white layer in the irregular margins, and numerous small colonies along the puncture line. The cultivations emit a disagreeable smell. Pathogenic to guinea-pigs and mice.

B. fluorescens liquefaciens, *B. ianthinus*, *B. erythrosporus*, *B. pyocyaneus* (See 'Colour-producing Bacteria,' p. 271).

Spirillum undula (Ehrenberg).—Isolated from infusions of putrefying animal matter. Rigid spiral thread 8 to 12 μ long, 1·1 to 1·4 μ broad. Has a long whip-like flagellum at each end, and progresses by rapid rotary movements.

Spir. concentricum (Kitasato). — Found in putrefying blood. Short spirillum with pointed ends, and two or three twists, each of which is 3·5 to 4 μ long, and 2 to 2·5 μ in diameter. In nutrient broth cultivations it develops into long spirals with from five to twenty twists. In stab cultivations it produces a cloudy growth on the surface extending into the depth of the gelatin, and on gelatin plates grows in concentric rings, alternately transparent and opaque.

B. proteus vulgaris.—Hauser regards this as the principal micro-organism in the putrefaction of dead animal tissues. It is normally present in the large intestine. It is a bacillus with rounded ends 0·6 μ broad, and varying greatly in length, sometimes being short and oval, and at others from 1·25 to 3·75 μ in length. In older cultivations it assumes many involution forms, appearing sometimes as filaments more or less wavy and spiral, and sometimes in forms resembling cocci, whence its name '*proteus*.' It is aërobic, has a flagellum, and is actively motile. Grown on gelatin it produces at first brownish or grey colonies, which afterwards assume curious shapes, and in the liquefied gelatin, appear as thick white cloudy masses. On agar-agar it forms a thick white layer. In nutrient media containing sulphur compounds it produces hydrogen sulphide and mercaptans. The products of the bacillus are poisonous to animals.

B. proteus mirabilis (Hauser, 1885).—This bacillus closely resembles the preceding, but its involution forms are more numerous, being spherical, pear-shaped, thread-like, etc. Grown on nutrient gelatin it forms a thick white layer, and causes slow liquefaction of the medium. In Hauser's experiments the injection of a sterilised cultivation into the peritoneal cavity of rabbits caused death.

B. proteus Zenkeri.—This was isolated by Hauser at the same time as the two preceding bacilli from putrid meat infusion. The bacillus is motile and varies greatly in size, but averages 1·65 μ in length, and about 0·4 μ in breadth. Grown on gelatin it produces after forty-eight hours small white colonies resembling the mycelium of fungus. It differs from the two allied species in not liquefying the gelatin.

B. coli communis.—This is a usual inhabitant of the large intestine.

In the typical form it is a short rod with rounded ends, about 2·5 μ in length, and 0·5 μ broad; but it sometimes resembles a micrococcus, and in cultivation filaments are also observed. It is motile and aërobic, but is also capable of developing in the absence of air. Grown on gelatin plates it produces greyish colonies more or less translucent. In stab cultivations the surface growth is dry, and may be either thin or thick and rugged. In the depths of the gelatin the colonies are white, and there is frequently a cloudiness near the surface. On the potato it produces a soft white growth which develops a brownish tint. This micro-organism has considerable resemblance to the bacillus of typhoid fever, from which it is only separated with great difficulty.*

The bacillus is pathogenic to rabbits and guinea-pigs. It is killed after five minutes at 66° C.

B. subtilis (the Hay Bacillus) is very widely distributed in the air, but develops most readily from an infusion of hay. It is a large bacillus, 4·5 to 6 μ in length, and about 4 μ broad, which occasionally forms filaments. Grown on gelatin, it produces a thick pellicle on the surface, and liquefies the medium. It is sometimes motile. It forms bright oval spores 1·2 μ long and 0·6 μ broad, which are not stained by ordinary aniline colours, and thus stand out in contrast to the stained bacillus.

Ascococcus Bilrothii.—Found by Bilroth in putrefying infusions of flesh. It forms characteristic colonies, consisting of oval masses with a tough surrounding envelope, in which are contained one or more groups of cocci 20 to 70 μ in diameter. It is an aërobic organism. The cultivations become strongly alkaline, owing to the liberation of ammonia. When grown on beetroot it forms a greenish-white slimy mass (Cohn).

B. cadaveris grandis (Sternberg). — According to Sternberg, this is one of a number of large anaërobic bacteria which produce the offensive gases in the putrefaction of animal matter. It is a large bacillus with an oval spore at one extremity, and is difficult to cultivate in artificial media. It is pathogenic to animals.

Bacterial Products of Putrefaction.—The anaërobic bacteria first decompose the albumin and fibrin of the flesh into albumoses and peptones, and then cause further disintegration, the nature of which depends to a large extent on the species of micro-organisms, and on the conditions as to temperature and access of air in the decomposing substance.

Among the decomposition products formed, the following may be mentioned:—Carbon dioxide, hydrogen, nitrogen, hydrogen sulphide, phosphuretted hydrogen, methane, ammonia, ammonium carbonate, various amines and amido-acids, different members of the fatty acid series, indol, skatol, ptomaines, etc. Many of the volatile substances are characterised by a disagreeable odour. The principal gases formed in the interior of a putrefying mass are methane, hydrogen sulphide, and hydrogen.

In the surface decomposition by the more aërobic bacteria the products formed are of a simple character, such as carbon dioxide and ammonia.

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* For a full discussion of the methods of distinction, see Stoddart (*Analyst*, xxii. p. 114).

Sapræmia, or 'Putrid Intoxication.'—That the products of many of the saprophytic bacteria are capable of producing serious disturbances when absorbed into the system of animals is well known. In the case of the bacteria of putrefaction it has been proved experimentally that many of the substances which they eliminate (ptomaines, etc.) are toxic to animals, whether introduced into the system by subcutaneous injection or by the mouth (cf. *Flesh Poisoning*, p. 222). Since the poisons which they elaborate are not destroyed at the usual temperatures of cooking, flesh which shows the slightest sign of decomposition must be regarded as dangerous.

It is uncertain whether the flesh of animals which have been poisoned by the products of putrefactive bacteria is itself dangerous. Ostertag considers that it cannot be so regarded provided *Septicæmia* is not simultaneously present. In support of his view he states that the flesh of poisoned animals is eaten every year without ill effects, and that the blood of the dead animals is not toxic on inoculation. He attributes this to the destruction of the poisonous substances by the action of the living cells.

The Bacillus of Sausage Poisoning (Botulism).

In December 1895 several persons in the Belgian village Ellezelles were poisoned through eating a ham, and four of the patients died. From the liver of one of them van Ermengem * isolated an anaërobic bacillus, the cultivations of which produced all the symptoms of sausage poisoning on inoculation into animals. Subsequently Brieger isolated from the cultivations a virulent toxine which is apparently the direct cause of the illness, since the micro-organism itself speedily perishes when introduced into an animal's system, being a simple saprophyte.

Bacillus botulinus can only develop under certain conditions in which the exclusion of air is complete, as in weak pickling fluids. From the poisonous ham in which the bacillus was first isolated no trace of ptomaines could be found, and the meat appeared perfectly sound. It was not found in other parts of the same pig or in the slaughter-house.

Characteristics.—*B. botulinus* forms rods 4 to 9 μ long with rounded ends and terminal spores. In appearance it has some resemblance to the bacteria of anthrax and of malignant œdema. Slightly motile. On gelatin it grows best at 20° to 30° C., producing slow liquefaction. At 38·5° the growth ceases in a few hours. The colonies are round and transparent.

Schneidemühl † points out that in 1889 Kempner isolated from swine fæces an organism identical in morphological and pathogenic properties with *B. botulinus*, and the toxine from which also produced these symptoms of botulism on inoculation. He regards this as an indication of the origin of van Ermengem's bacillus (*cf.* p. 226).

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* *Trav. du Lab. d'Hyg.*, Ghent, 1897.

† *Cent. f. Bakt.*, 1898, p. 577.

Pathogenic Bacteria.

Pyogenic Bacteria.—As a rule, one or more micro-organisms are found in pus. It has, however, been abundantly proved that bacteria are not essential to the formation of abscesses, which may also be caused by chemical agents, such as turpentine and croton oil. Of the numerous micro-organisms which under ordinary circumstances are the cause of suppuration, those most frequently met with are *Staphylococcus pyogenes aureus* and *Streptococcus pyogenes*, then *Staph. pyogenes albus*, and less frequently *Staph. cereus flavus*, *Staph. cereus albus*, *Staph. pyogenes citreus*, *Micrococcus tenuis*, *Bacillus pyogenes fœtidus*, and *M. tetragonus.* Many other organisms associated with various diseases, such as the streptococcus of erysipelas, also give rise to suppuration on inoculation.

Staph. pyogenes aureus. — This is a very common and widely distributed saprophytic organism, which is found normally on the body, and especially on mucous surfaces. A spherical micrococcus 0·7 μ to 0·9 μ in diameter, which occurs singly, in pairs or groups, and sometimes in chains of three or four individuals. Aërobic and facultatively aërobic. Grown on gelatin it forms golden colonies where it comes in contact with the air, and causes liquefaction. The cultivations, especially those on potatoes, have a sour smell. According to Sternberg it perishes after ten minutes at 56° to 58° C.

Staph. pyogenes albus (Rosenbach, 1884). — Closely resembles the preceding organism in size and morphological characters, but the surface cultivations are white. Flügge states that it is more frequently met with in the lower animals than *S. pyogenes aureus.* It causes the liquefaction of gelatin.

Staph. pyogenes citreus (Passet, 1885). — This micrococcus is indistinguishable in form from the two preceding organisms. It differs from them in forming a lemon-yellow growth and in liquefying gelatin more slowly.

M. pyogenes tenuis (Rosenbach, 1884).—Micrococci of irregular size, somewhat larger than the preceding species. Grown on agar-agar produce thin, transparent, shiny colonies.

Strept. pyogenes (Fehleisen, 1883).—Spherical cocci 0·4 to 1 μ in diameter, multiplying by division in one direction. Aërobic and facultatively anaërobic, non-liquefying. Grown on gelatin, form small white colonies. No growth on potato. Thermal death-point (Sternberg), 52° to 54° C. for ten minutes.

Pyæmia.—This is an old term applied to those cases in which the toxic bacterial products are absorbed into the system from the abscess, so that the blood-poisoning becomes general instead of local. Sternberg includes it under the wider term *toxæmia*, which he applies to all cases in which bacterial products (as distinct from the bacteria) produce general disturbances at a distance from the seat of infection, as, for example, in the case of tetanus and diphtheria.

The Flesh of Infected Animals.—Where pyogenic bacteria are actually present Ostertag considers that there can be no question as to the flesh being dangerous. This was confirmed by Karlinski, who gave milk containing *S. pyogenes aureus* to forty-eight animals, and produced general infection in six, and local abscesses in other cases. Moreover, numerous cases have been recorded of poisoning through eating flesh of animals with general pyæmia.* According to Fischoeder,† when the disease is circumscribed the flesh, after removal of the affected parts, may be freely sold, but when there is emaciation, and the flesh is watery, it should be excluded from the market. The flesh is dangerous when the abscesses are not circumscribed, and when the animal, before slaughtering, showed signs of fever and extreme debility, etc.

Septicæmia.—Under this title are grouped a number of acute organic disturbances, caused by the presence and development of bacteria in the blood, causing a general infection, which usually terminates fatally within one or two days. The general symptoms of the infection, whether by accidental or artificial inoculation with the bacteria, are fever, enlargement of the spleen, congestion of various organs, and hæmorrhage. The micro-organisms are found in the blood and tissues throughout the body.

Bacteria producing Septicæmia in Animals.—

1. *The Bacteria of the Rabbit Septicæmia Group.*

According to Baumgarten, the micro-organisms which produce rabbit septicæmia, fowl cholera, swine fever (*Schweinseuche*), the epidemic disease of deer and of cattle (*Wildseuche* and *Rinderseuche*), and of buffaloes (*Buffelseuche*), have the same morphological, biological, and pathogenic properties. Federn regards them as varieties of the same species, and Sternberg ‡ agrees with Caneva (1891) in regarding them as identical, and describes them under the name of *B. septicæmiæ hæmorrhagicæ*. Klein,§ however, considers that more work is required before this can be regarded as proved.

Bacillus septicæmiæ hæmorrhagicæ. — Small bacilli with rounded ends 1·4 μ long, and 0·6 to 0·7 μ broad, occurring in pairs or in chains of 3 or 4. Aërobic, non-motile and non-liquefying, forming small white colonies on gelatin. Stain more readily at the extremities than in the centre, so that after short staining the rods have the appearance of a diplococcus.

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* Ostertag, *Der Fleishchbeschau*, p. 475.
† *Leitfaden der Fleischbeschau*, p. 159.
‡ *Bacteriology*, 1896, p. 429.
§ *Micro-organisms and Disease*, p. 223.

2. *The Bacillus of Hog Cholera. Cf.* p. 282.
3. *The Bacillus of Swine Erysipelas. Cf.* p. 283.
4. *B. pyogenes fœtidus. Cf.* p. 276.
5. *B. enteritidis. Cf.* p. 275.
6. *The Bacillus of Grouse Disease* (Klein, 1889).—Aërobic, non-liquefying, non-motile bacillus, with rounded ends, 0·8 to 1·6 μ long. Also as spherical or oval cells, 0·6 μ long and 0·4 μ broad. Occurs singly or in pairs, or in chains of 3 or 4. Grows on agar-agar at 36° to 37° C., forming a dry, thin, grey layer. It was found in the lungs and liver of grouse which had died of an epidemic disease.

Rabbit Septicæmia.—The micro-organisms producing septicæmia in rabbits (Koch) are oval cocci, 0·8 μ long and 0·6 μ broad. They were found in the blood after the injection of putrid meat infusion. Grown on gelatin they form round white colonies. According to Ostertag,* the bacteria usually perish after fifteen minutes at 55° C., or ten minutes at 80° C. But in order to destroy them in flesh, even in thin slices, the temperature must be kept for at least an hour at 80° C.

The Bacilli of Davaine's Septicæmia in Rabbits.—These were found in the blood of rabbits into which Davaine had injected putrid ox blood. They are non-motile, short, oval rods, 1·5 μ long and 0·8 μ thick. On gelatin they form small white circular colonies. In stained preparations the granules at each end are stained more readily than the centre of the rod.

Epidemic Disease of Deer and Cattle (*Wildseuche and Rinderseuche*).—This is an epidemic disease which attacks cattle (*Rinderseuche*), deer (*Wildseuche*), and horses. In the pectoral form of the disease, which is the most common among deer, there is acute pleuro-pneumonia and hæmorrhage into the breast cavity.

The Bacilli of 'Wildseuche.'—These are small motile rods with rounded ends, 1·0 to 1·4 μ long and 0·4 to 0·7 μ broad.

The Flesh of Infected Animals.—Notwithstanding the resistance of the bacilli to the action of the gastric juice, there is no evidence to show that the flesh of infected animals is injurious to man, and in fact such flesh has repeatedly been eaten without ill effects (Friedberger). In Bavaria, however, the sale of the raw flesh is prohibited.

Swine Fever (*Schweinseuche*).—This disease (like *Wild-* and

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* *Handbuch der Fleischbeschau*, p. 439.

Rinderseuche, p. 281) can occur in three forms—exanthematic, pectoral, and intestinal. The skin becomes bright red, especially on the neck, abdomen, and breast, and the animals have difficulty in breathing, and a cough. In the acute form the symptoms resemble those of swine erysipelas, and in the chronic cases those of tuberculosis. About 50 per cent. of the animals attacked succumb. The disease is highly infectious, and can be contracted by inoculation, by respiration, and by introduction with the food.

The Bacilli of Swine Fever.—These are motile bacilli, with a great resemblance to those of fowl cholera, but with a tendency to unite in longer threads. Moreover, the bacilli of swine fever are harmless to hens and pigeons (Itzerott and Niemann). Grown on gelatin they produce scanty white colonies, without liquefaction. They are readily stained with aniline colours, but not by Gram's method.

The Flesh of Infected Swine.—Fiedler and Bleisch * regard the flesh as dangerous to man, and Zchokke † records a case of poisoning in 1897 with ham, from which bacteria, which agreed in morphological and biological characteristics with those of swine fever, were isolated, and which, inoculated into rabbits, produced death. Ostertag, however, points out that the general experience of meat inspectors leads to the opposite conclusion. Moreover, since the bacilli perish after an hour at 80° C., he considers that thorough cooking of the flesh would remove all risk. By German law it is allowed to be sold, provided it be first well cooked.

Hog Cholera (*Swine Plague, Schweinepest*).—This disease had its origin in America, where it is very fatal to swine. It was introduced from America into England, and subsequently on to the Continent of Europe. In Germany it is not very frequently met with. It takes either an acute or chronic form, and is characterised by septicæmia and hæmorrhage upon the mucous membranes. The *post-mortem* appearance of the spleen is enlarged, soft, and dark.

The Bacilli.—Small, actively motile rods with rounded ends, 1·2 to 1·5 μ long and 0·6 to 0·7 μ broad, which usually occur in pairs. Aërobic (facultatively anaërobic) and non-liquefying. Grown on gelatin they form deep spherical colonies. Yellowish-white growth on potato. Novy isolated from pure cultivations a toxine '*suso-toxine*,' which produced death when inoculated into animals.

The Flesh of Infected Animals.—There is no evidence that the flesh of swine which have suffered from hog-cholera has ever caused illness in man. From some experiments by Smith it appears that bouillon cultivations of the bacilli are sterilised in fifteen minutes at 70° C., and in an hour at 54° C. Sternberg states that the bacilli can resist desiccation for nine days to a month, according to the thickness of the layer dried. By a German law of July 9, 1894, the sound flesh of infected animals may be sold, with a declaration as to its nature, after thorough cooking. The affected parts must be burned or buried.

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* *Der Fleischbeschau*, p. 455.

† *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 189.

Fowl Cholera.—The birds are suddenly attacked, the symptoms being diarrhœa, fever, drowsiness, hæmorrhage in the viscera, and death in twenty to forty-eight hours.

The Bacillus of Fowl Cholera.—This was discovered by Pasteur in 1880. It is a small short rod 1 to 1·2 μ in length. In pure cultivations two or more rods are found lying together, and in many of them bright spots can be observed, which however are not spores (Itzerott). It is aërobic and non-motile. In gelatin stab cultivations it produces a thin white streak without liquefaction. On potato and agar-agar a yellowish-grey thick surface growth is formed. It is characteristic of this organism that it is only stained intensely at the ends with a basic aniline colour if the action of the dye is not continued too long. It is not stained by Gram's method. It is pathogenic to many birds, by subcutaneous injection or through the mouth, and also to mice and rabbits. Guinea-pigs are only locally affected, and the same applies to horses and sheep.

The Flesh of Infected Birds.—From experiments of Perroncito and Kitt it appears that dogs and cats devour the infected flesh without injurious results.

Swine Erysipelas (*Rotlauf*).—Klein * states that 60 per cent. of swine attacked by this disease die. The time of incubation is from three to four days, and in a few hours after the animal has shown signs of fever, red patches make their appearance on all the smoother parts of the skin, such as the neck, ears, and abdomen. These gradually become darker and extend until the whole body is covered. The spleen, liver, kidneys, and heart are all affected, and death ensues in twenty-four to forty-eight hours.

The Bacilli of Swine Erysipelas.—These have the form of rods 0·8 to 1·5 μ long and 0·1 to 0·2 μ broad. They are found in the secretion of the lymphatic glands and in the blood of the diseased animals. Grown on gelatin they form a silvery-grey layer without liquefaction. On agar-agar they produce a soft grey skin. There is no growth on potatoes. Spore formation has been observed. The bacilli are pathogenic to rabbits, pigeons, and mice, but cattle, horses, guinea-pigs, and hens are refractory.

Influence of Heat and Preservatives on the Bacilli. — Pure cultivations of the bacilli are destroyed after five minutes' heating at 55° C. Petri * found that the ordinary methods of cooking meat did not kill those in the interior of the flesh. By salting and pickling, their vitality was weakened, but they did not die until after a month. In another experiment they were found to be still virulent in flesh which had been salted for a month. In smoked ham they were found capable of infecting after three months, but were dead after six months.

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* *Micro-organisms and Disease*, p. 252.

The Flesh of Infected Animals.—The bodies of infected swine do not, as a rule, stiffen much (*rigor mortis*), and the flesh rapidly decomposes. Ostertag states that it has been abundantly shown that the fresh flesh is not injurious to man.

Malignant Œdema.—The cause of this disease was first made known by Koch, who produced it in guinea-pigs by subcutaneous inoculation with garden earth. It can be communicated to horses, dogs, sheep, calves, goats, and swine, and according to Ostertag occurs spontaneously in horses. Arloing and Chauveau stated that cattle were immune, but Kitt found that inoculation with the bacteria produced pronounced local symptoms in them.

The disease is characterised by the formation of an extensive œdema near the seat of infection, followed by mortification of the surrounding tissues. The large intestine is often inflamed, and the spleen and other organs congested. Death results in from twenty-four to forty-eight hours.

The Bacilli.—These are found in the spleen and in the pus. They are long rods 2·5 to 3 μ in length and 1 μ broad, with rounded ends, and occur singly, in chains, or in filaments. They are motile and anaërobic, and in stab cultivations form deep-seated globular colonies which eventually sink to the bottom as white masses in the liquefied gelatin. When cultivated on agar-agar the colonies produce bubbles of gas. Oval spores are formed either at one end or in the middle of the rod. In cover-glass preparations the bacilli are not unlike the bacilli of anthrax.

The Flesh of Infected Animals.—There is no proof that the flesh of animals which have suffered from malignant œdema is injurious to man. But Lehmann † considers it dangerous to eat both in the raw and cooked condition; whilst, since the disease is communicable to man, there is always the risk of infection through any cut or abrasion of the skin.

Tetanus.—The characteristic symptoms of this disease are spasmodic contractions of the muscles, which is usually caused by the contamination of a wound by the specific bacillus. It occurs most frequently in horses, but is also common in new-born lambs from infection of the navel wound.

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* Ostertag, *loc cit.*, p. 447.

† *The Methods of Practical Hygiene*, ii. p. 32.

Bacillus Tetani.—The micro-organism producing tetanus was discovered by Kitasato in 1891. It is frequently met with in soil or dust. It is a slender straight rod, which forms a large spore at one extremity, giving it a characteristic drumstick appearance. It is anaërobic, and growing the depths of the gelatin, where the oxygen is absent, in a radiating form, and after a considerable time liquefies the gelatin. The cultivations develop a strong odour, and in the gases evolved hydrogen sulphide and mercaptans can be detected. It can readily be stained with aniline colours or by Gram's method. The spores can be stained red in contrast to the bacillus, which is stained blue by the Ziehl-Neelsen method, and methylene blue.

The Flesh of Infected Animals.—From the experiments of Sormani,* who fed animals with pure cultivations of the tetanus bacillus, the flesh of animals infected with tetanus would seem to be harmless when eaten. It was found that the digestive apparatus could withstand a dose 10,000 times greater than was fatal in injections. This conclusion is the more probable since the bacilli must frequently be eaten by cattle with their food. At the same time the flesh is not normal, since there is unusual whiteness, degeneration of the muscular tissue, defective bleeding, and a characteristic sickly odour.

Influence of Heat on the Virus.—Kitasato found that the toxine of the tetanus bacillus (tetanine) was rendered completely harmless after five minutes at 65° C., so that all chance of infection would be destroyed by thorough cooking of the flesh.

Rabies (*Tollwuth*).—There is no evidence to show that hydrophobia can be communicated by eating the flesh of animals which have been infected with rabies, but it should be excluded from the market, since there is a possibility of infection through handling it.

The action of gastric juice on the virus was made the subject of experiments by a Russian doctor, Wyrsykowski.† Starting from the fact that the flesh and brain of a mad animal did not produce illness in animals eating them, he placed the *medulla oblongata* of an infected rabbit in a thermostat with artificial gastric juice. Of twenty-one rabbits inoculated with the artificially-digested virus none became ill, whereas seventeen inoculated with the fresh (undigested) virus became infected.

From Sternberg's experiments,‡ it appears that the virus is rendered harmless after being heated for ten minutes at 60° C. The primary cause of the disease has not yet been determined notwithstanding the researches of Pasteur and others. Various micro-organisms have been described in connection with rabies, but pure cultivations of these have not infected animals on inoculation. The brain, spinal cord, and nerves appear to be the principal seats of the virus.

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* Ostertag, *Der Fleischbeschau*, p. 361.

† Ostertag, *loc. cit.*, p. 373.

‡ Sternberg, *Bacteriology*, p. 521.

Glanders. — *Occurrence.* — This disease is almost peculiar to horses, asses, and mules, though it can be communicated to man. The horse, goat, ass, and sheep have been infected with artificial cultivations, but cattle, swine, and mice are immune.*

Mode of Infection.—Primary intestinal infection has not been observed in the case of any animal, and infection usually occurs through external abrasion of the skin.

Effects.—Glanders is characterised by the formation of nodules and tumours. There is frequently ulceration of the nostril, but no characteristic alteration of the muscular tissue.

The Bacillus.—Löffler and Schulz in 1882 discovered that glanders was produced by a bacillus (*B. mallei*). It is a non-motile aërobic rod 1·5 to 3·5 μ in length, with some resemblance to the tuberculosis bacillus, but rather thicker. Spore formation is not known to occur.

Grown on agar-agar (at 35° to 38° C.) it produces a moist white layer, and on blood serum a white film without liquefaction. It forms a characteristic golden growth on sterilised potato, which gradually darkens and assumes a reddish colour. It sometimes forms filaments in cultivations.

Staining.—Löffler employed an alcoholic solution of methylene blue. Gram's method cannot be used.

Influence of Heat.—The glanders bacillus in pure cultivations perished at 60° to 70° C.

The Flesh of Glandered Animals.—By a German imperial edict the bodies of all animals infected with glanders must be destroyed without breaking the skin. It has, however, been shown that the flesh of glandered animals can be eaten with impunity, as Decroix relates was the case in the siege of Paris. Feeding experiments have given negative results, but there is always the possibility of infection in the handling flesh if there is any abrasion of the skin.

The Mallein Reaction. — This is used to detect glanders in horses in the same manner as the tuberculin test for cattle. A broth cultivation of the bacilli of glanders is left for a month at 37° C. It is then sterilised for thirty minutes at 100° C., evaporated to one-tenth of its volume on the water-bath, and filtered through paper. The brown liquid thus obtained is the crude *mallein*. When treated with several volumes of alcohol a precipitate is obtained which consists of a mixture of several active principles (Nocard). The injection of crude mallein (0·25 c.c.) into a glandered horse causes an intense reaction, the temperature often rising from 3° to 4° after twenty-four hours, whereas healthy animals are hardly affected by the same dose. Man is extremely sensitive to the test. In practice the mallein is diluted with a weak solution of phenol, as in the tuberculin test (p. 292).

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* Itzerott and Niemann, *Mikrophot. Atlas der Bakterienkund.*, p. 64.

Anthrax.—(*Charbon, Pustule Malignant, Splenic Fever, Woolsorters' Disease, Milzbrand.*)

Occurrence.—This highly infectious disease may occur in all the domestic animals and in man. The sheep is the most susceptible, then the ox, and then the horse, whilst the pig has considerable powers of resistance. Wild animals, birds, and frogs (kept at a suitable temperature) can also be infected. It is endemic in certain parts of Germany and France, and has been introduced into this country through spores attached to the skins of the animals.

Mode of Infection.—This is usually by inoculation from surface wounds, but sometimes occurs by inhalation and by entry into the alimentary canal, especially in the case of the spores.

Symptoms.—The muscular tissue, especially that of the abdomen, becomes pale and rotten, and there is hæmorrhage in different organs. But the most characteristic symptom is the enlargement of the spleen, which assumes a blackish-red colour.

The Bacilli. — On examining the blood or spleen of the diseased animal under the microscope, numerous motionless rods can be observed. These are the *Bacilli anthracis*, short cylindrical rods with square-cut ends, 3 to 6 μ long and 1 to 1·5 μ broad. The filament form does not occur in the living animal. When grown on gelatin plates grey dots appear which after two or three days develop into colonies of a considerable size, and the filamentary structure can be readily made out. The colonies become depressed in the centre and liquefaction commences. In stab cultivations the growth forms a white line with horizontal feathery projections. Liquefaction commences at the surface, and when complete the colony sinks to the bottom as a white flocculent mass.

Spores are only produced at a suitable temperature (18° to 34° C.). Neither in living animals nor in undecomposed dead bodies are spores (as a rule) produced.

Hanging-Drop Cultivation.—A drop of nutrient broth is inoculated with a trace of the blood of the animal, and placed on a cover glass. Over this is placed a hollowed-out glass slide, the edge of the hollow having been painted round with vaseline. The slide is then inverted, and the development of the bacilli can be observed under the microscope.

Staining.—Cover-glass preparations of blood, etc., may be stained by treatment with Neelsen's solution and alcohol.

The spores are best observed by double-staining with Ziehl-Neelsen solution and methylene blue. Sections of tissue are stained by Gram's method.

Action of Heat on Anthrax Bacilli.—The bacilli are rendered harmless in 10 to 15 minutes at 55° to 60°, but a longer time is necessary for the destruction of the spores.

The Flesh of Animals Infected with Anthrax.—Bollinger * contends that the disease is not so readily communicated to man by eating the flesh of animals that have suffered from anthrax as has been supposed, and this view has been confirmed by others. Behring, for instance, records a case in which the flesh of a bull was eaten without ill effects, although the two men who slaughtered the animal became infected with anthrax. Ostertag therefore considers that as a general rule the flesh might be eaten with impunity, for the bacilli, without spores, are destroyed by the gastric juice, and spores are not usually present in fresh meat. But such flesh must, notwithstanding this, be regarded as dangerous, for mere handling of it has been known to cause infection. Schmidt-Mulheim, too, has shown that spores can form under favourable conditions (*e.g.*, high temperature), and these might cause intestinal infection (*Darmmilzbrand*).

Perroncito has described a disease which occurs in Sardinia, affects horses, asses, cattle, and swine, and is communicable to man. It resembles anthrax in many of its symptoms, but is caused by another micro-organism, *B. proteus virulentissimus.*

Quarter Evil.—(*Symptomatic Anthrax*, *Charbon Symptomatique*, *Rauschbrand.*)

Occurrence and Effects.—This is a disease with a slight resemblance to anthrax. It spreads rapidly among cattle and sheep, but swine and poultry are immune (Itzerott and Niemann). Large tumours are formed under the skin, containing a dark-coloured liquid. It originates from deep injuries of the skin or mucous membrane. The muscles adjoining the seat of infection are of a brown or black colour.

The Bacillus. — This was discovered by Feser and Bollinger in 1876. It is a motile rod 3 to 6 μ long and 1 μ broad, which becomes motionless after the formation of its large terminal spores. It is an anaërobic organism. In gelatin stab cultivations it forms round white globules, the surrounding gelatin is liquefied, and there is an evolution of gas.

The Influence of Heat.—The bacilli have great powers of resistance to the action of heat. They are still virulent after an hour at 80° C., but are killed in five minutes at 100° C. in a steam apparatus. The experiments of Kitt † showed that the spores in dried flesh are not destroyed but only weakened in a current of steam. Fresh flesh containing the bacilli can be boiled for half-an-hour, and dried flesh-powder for six hours, without being sterilised.

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* Ostertag, *loc. cit.*, p. 365.

† *Ibid.*, p. 442.

The Flesh of Infected Animals.—Ostertag states that the flesh can be eaten without ill effects, but it rapidly undergoes putrefaction (Feser), and, on keeping, develops a rancid odour somewhat resembling that of smoked herrings (Kitt).

Foot-and-Mouth Disease (*Apthenseuche*).—*Occurrence and Effects.*—This is an infectious febrile disease which attacks sheep cattle, and pigs. It is characterised by the formation of bladder-like vesicles affecting the mouth and feet.

The Micro-organism.—This has not yet been identified with certainty, though several bacteria have been described as the cause of the disease. Piani and Fiorentini, from their experiments on the liquid from the vesicles, are of opinion that it is produced by protozoa.

The Flesh of Infected Animals.—Since the disease can be communicated to man, parts containing vesicles must be regarded as dangerous, but from the results of experiments, flesh free from vesicles appears to have no ill effects.

A case is recorded in which an attendant in a slaughter-house in Dresden became infected with the disease through smoking a cigar which he had handled after touching an infected carcass.*

Cow-Pox and Sheep-Pox.—These are acute febrile diseases characterised by the formation of vesicular pustules. The micro-organisms producing the diseases are not known with certainty. They are distinct diseases, cow-pox being conveyed by inoculation and not being infectious, whilst sheep-pox is infectious.

Tuberculosis.—*Occurrence.*—In one or other of its forms tuberculosis is widely distributed among domestic animals. It is common in the ox and cow, fairly common in swine and poultry, but rare in sheep, goats, horses, cats, and dogs.

Effects.—The disease may be either local or general. When the lungs are affected small oval or spherical tubercles are found, some firm and caseous, others containing pus. At a later stage large oval cells ('giant cells') with several nuclëi appear. In the advanced stage of the disease the animal becomes emaciated, the flesh watery, and the fat disappears.

Mode of Infection.—This is caused by inhalation or by intestinal infection.

The Bacillus.—The bacilli of tuberculosis (discovered by Koch in 1882) are straight or curved non-motile rods from 2 to 4 μ in length. They frequently have a beaded appearance, and occur either singly, in pairs, or in groups. Spore formation occurs in old colonies. Grown on solidified blood serum (37° to 38° C.) they form small, thick, white scales. On glycerin agar they produce little nodules, coalescing in time into a cauliflower-like mass.

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* *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 18.

Staining.—The tuberculosis bacillus has a characteristic staining reaction, which it shares with the leprosy bacillus. It is stained with fuchsin, warmed for ten minutes, and decolorised with dilute mineral acid. When stained it often shows a beaded appearance, which is probably caused by the breaking up of protoplasm. Sections of tissue may be stained by the following method (Crookshank):—(1) Warm Neelsen's solution on the sand-bath till it steams. (2) Stain for five or ten minutes. (3) Rinse in water. (4) Decolorise in dilute hydrochloric or sulphuric acid. (5) Rinse in water. (6) Counter-stain in methylene blue for three minutes. (7) Alcohol. (8) Oil of cloves. (9) Mount in Canada balsam.

In Koch's method the section is stained for an hour at 40° C. in a mixture of water 200 c.c.; alcoholic methylene blue, 1 c.c.; 10 per cent. potassium hydroxide, 0·2 c.c. It is then washed with water, counterstained in an aqueous solution of Bismarck brown, washed, and mounted.

Action of Heat on the Bacillus of Tuberculosis.—From experiments on pure cultivations Bang found that the bacillus died at 85° C. According to Jersin it perishes after ten minutes at 75° C., but can withstand a temperature of 65° C. Ordinary methods of cooking are, therefore, probably insufficient to destroy the bacilli in the middle of the flesh. Forster has determined the thermal death-point of the bacilli in milk:—55° C. for four hours; 60° for one hour; 65° for fifteen minutes; 80° for five minutes; and 95° for one minute (*cf.* p. 212).

Influence of Salting and Smoking.—The bacillus is very resistant to the action of salt. Thus, Forster found that pure cultivations covered with salt were capable of infecting after two months, and that even salting and subsequent smoking did not destroy their virulence.

*Influence of Gastric Juice on the Bacilli.**—Falk first showed the powers of resistance of the bacilli to the action of artificial gastric juice. Strauss and Würtz found that they retained their power of infecting for six hours in natural gastric juice, and only perished after twenty-four hours. Zagavi established the facts that tuberculosis bacilli kept in artificial gastric juice at 38° C. were still virulent after three or four hours. After seven to nine hours they only caused local tuberculosis, and after eighteen to twenty-four hours were no longer infectious. Wesener also

* Ostertag, *loc. cit.*, p. 385.

showed by feeding experiments that small quantities of pure cultivations produced no effect on animals, that larger quantities caused tuberculosis of the mesentery glands, and that only after repeated feeding with large quantities did tuberculosis of the intestines, liver, and spleen occur.

Influence of Putrefaction.—Galtier found that the bacilli were still virulent after having been left in putrefying media for twenty-four days. From the experiments of Schottelius and of Gaertner, they appear to be capable of infecting after being left with the decomposing matter for months in the earth.

The Flesh of Tuberculous Animals.—Ostertag * summarises the experiments which have been made with the flesh of animals which had suffered from tuberculosis. Nocard experimented with the flesh of twenty-one cows with general tuberculosis, but only in four cases were guinea-pigs infected. Galtier (1891) found that the flesh-juice of tuberculous animals may contain the bacilli, but that this is not the rule. In fifteen experiments the disease was only twice communicated. In other experiments animals were given as much of the raw flesh of tuberculous animals as they could eat, but in no case was tuberculosis produced. Hence Galtier came to the conclusion that there is no great danger in the flesh, provided the infected organs are destroyed.

Forster experimented with the finely divided flesh of highly tuberculous animals and obtained three positive results. In Bang's feeding experiments with the blood there were only two cases of infection to nineteen negative results. In his opinion there is no risk in eating the flesh so long as the disease was unmistakably local. Kastner obtained negative results in twelve experiments, in which the flesh-juice was injected into the peritoneal cavity of animals.

Professor Thomassen of Utrecht, in the report to the Congress on Tuberculosis, 1898, described feeding experiments with the flesh of animals with general tuberculosis. Ten pigs were fed for three months on the raw meat, each consuming three to fifteen kilos. Seven remained healthy, while three became tuberculous. As the meat contained fragments of bone, it is possible that in these cases small abrasions were produced in the mucous membrane of the mouth and stomach, and that the disease was thus introduced directly into the system.

Ostertag considers that, as a rule, the flesh and flesh-juice of tuberculous animals is not infectious, since it either contains no bacilli or too few. Only when the disease is in a very advanced stage does the flesh appear to be infectious. Flesh must, therefore, be regarded as dangerous in all cases of general tuberculosis affecting the muscles, bones, and lymph glands, and the flesh of emaciated tuberculous animals must be regarded as unfit for food, without reference to the tubercular processes.

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* *Loc. cit.*, p. 403.

The British Commissioners on tuberculosis appointed in 1896, recommended in their report, issued in 1897, that the entire carcass and organs should be seized—

(*a*) When there is miliary tuberculosis of both lungs.
(*b*) When there are tuberculous lesions on the pleura and peritoneum.
(*c*) When tuberculous lesions are present in the muscular system or in the lymphatic glands embedded in or between the muscles.
(*d*) When there are tuberculous lesions in any part of an emaciated carcass.

The entire carcass is not condemned, but all parts of it infected with tubercles are to be seized when—

(*a*) The lesions are confined to the lungs and lymphatic glands.
(*b*) The lesions are confined to the liver.
(*c*) The lesions are confined to the pharyngeal lymphatic glands.
(*d*) The lesions are confined to any combination of the foregoing, but are collectively small in extent.

In France the flesh is condemned when the tuberculosis is general, or when the chest wall or abdominal cavity or the greater part of an organ is affected.

The Tuberculin Test.—This is based on the fact that when from 3 to 4 c.c. of a freshly-prepared 10 per cent. solution of tuberculin (1 c.c. of crude tuberculin dissolved in 9 c.c. of a 0·5 per cent. aqueous solution of phenol) is injected into the cellular tissue of the neck of cattle there is only a slight rise of temperature (0·5 to 0·8° C.) in the case of a healthy animal, whereas with tuberculous animals the rise in temperature is marked. When it amounts from 0·8 to 1·4° C. the animal is regarded as suspicious, and the test repeated in a month's time.

Besson * gives a description of the method used in the Pasteur Institute (1898) for the preparation of tuberculin:—A pure cultivation of avian tuberculosis (which grows more rapidly than the human variety) is sterilised at 100° C., concentrated to a tenth of its volume on the water-bath, and filtered through paper. The filtrate, which is a brown syrup, has a sweet, characteristic odour and constitutes the crude tuberculin. It can be purified by precipitation, but this involves the loss of a large proportion of the tuberculin.

Animals differ greatly in their power of resisting inoculation with tuberculin. An injection of 10 c.c. of the crude tuberculin into healthy dogs or cattle produces no symptoms beyond the slight rise in temperature. Guinea-pigs and rabbits are more susceptible, the former withstanding only 2 c.c. and the latter 5 c.c. Man is extremely susceptible, and an inoculation of only 0·25 c.c. causes fever (102° F.), diarrhœa, and vomiting. Stronger doses than those ordinarily used in the tuberculin test are inadvisable, since they may be followed by fatal results in the case of tuberculous cattle.

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* *Technique Microbiologique et Serotherapique*, 1898, p. 443.

At the fourth Congress on Tuberculosis which was held in Paris in 1898, Professor Bang described the precautions taken by the Danish Government to isolate diseased animals so as to check the spread of the disease. By a Statute of the Government (1893) the owners must submit their cattle to the tuberculin test, and where a reaction is obtained the cattle must be isolated as completely as possible. Similarly in the case of imported cattle, the animals are kept in quarantine until they have been tested, and those which react are slaughtered.

At the same Congress Bang gave the following statistics of the number of animals found to be tuberculous in the slaughter-houses of different countries*:—

			Per Cent.
GERMANY.—	Baden,		3·67
	Bavaria,		5·0
	Hamburg,		8·56
	Prussia,		12·7
	Saxony,		27·5
	Zurikan,		37·5
AUSTRIA.—	Vienna,		1·3 to 1·8
FRANCE.—	Toulouse,		9·28
	Brie and Beauce (Nocard's Statistics),		25·0
HOLLAND.—	Amsterdam,		8·12
	Rotterdam,		7·0
ENGLAND.—	Manchester,		29·4
DENMARK.—	Copenhagen	1895,	29·66
		1896,	25·31
		1897,	26·87

In regard to reactions to the tuberculin test there were

	Positive Reactions. Per Cent.
In Bavaria, 1895 and 1896,	37·2
„ Vienna,	39 to 43
„ Strebel,	41 to 52·5
„ Copenhagen,	28·8
„ Sweden,	42·2, 46·9

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* *B. M. J. Epitome*, 1898, p. 26.

The Bacilli of Tuberculosis in Cold-Blooded Animals.—Battaillon, Dubard and Terre * found in a large tumour in a carp numerous giant-cells containing bacilli with the same morphological and staining properties as the ordinary bacilli of tuberculosis, but growing best at a temperature of 23° to 25° C. On inoculating carp with the pure cultivation no illness was produced, but the bacilli could be detected in the inoculated animal after a month. Inoculation into frogs caused death in nineteen days with occasionally tubercular lesions of the lungs and liver. By inoculating frogs with tuberculous material from a guinea-pig, bacilli which grew at the usual temperature could subsequently be isolated. It was not possible, however, to communicate tuberculosis to guinea-pigs or pigeons with these cultivations. The conclusion arrived at was that the bacilli of human or avian tuberculosis may be converted into a saprophytic form by being passed through the body of a cold-blooded animal.

Pleuro-Pneumonia of Cattle (*Lungenseuche*).—This is a lung disease of cattle, characterised in acute cases by difficulty of breathing, fever, etc., while in chronic cases there are often no external signs. As a rule only the left lung is affected, and after death it shows signs of inflammation and is more or less solid. The disease appears to be only communicated by exposure of the cattle to the exciting cause, and has not yet been conveyed artificially from one animal to another.

Several micro-organisms have been described as the cause of pleuro-pneumonia. Arloing isolated four, one of which was a bacillus and the others cocci, from the lungs of a diseased animal. The *Pneumo-bacillus liquefaciens bovis* was a short non-motile rod, which when cultivated on potato formed a white growth, sometimes becoming brown and sometimes green. Nocard, however, does not accept this bacillus as the cause of the disease, and Crookshank † considers that the micro-organism has yet to be discovered.

The Flesh of Infected Animals.—Pleuro-pneumonia of cattle does not appear to be conveyed to man, and Fischoeder regards the flesh as harmless. In Germany it is allowed to be sold after being cooked and left until perfectly cold. The lungs, however, must in every case be destroyed. In the early stages of the disease the flesh appears normal in consistence and colour, but later on it becomes emaciated, discoloured and flabby. In England it is customary to condemn only those carcasses in which the muscular tissue shows such signs of disease.

Rinderpest (*Cattle Plague*).—This disease is only met with in cattle under natural conditions, although it can be communicated artificially to other ruminants. Among the symptoms are fever and acute inflammation of the intestine. Red patches appear on all the visible mucous surfaces, which are subsequently covered with a greyish white deposit. Death usually occurs in from four to seven days. In many of its symptoms rinderpest resembles smallpox, although the diseases are quite distinct (Crookshank). The cause of the contagion is unknown.

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* *Zeit. Fleisch u. Milch Hyg.*, 1898, p. 151.

† *Bacteriology*, p. 243.

The flesh of infected animals is not dangerous to man, but it is usual to dispose of the entire carcass of the animal to prevent the disease spreading among other cattle.

Calf Diphtheria.—Under this name Damman described a disease with a strong resemblance to human diphtheria. Löffler, however, showed that the necrotic deposits formed in the mouths of calves were different from those in human diphtheria, and that the disease was due to long filamentary bacilli.

As no experiments have been made to determine whether the flesh of the diseased calves is injurious to man, the question is still an open one.

Fowl Diphtheria.—According to Löffler the 'diphtheritic' formations in the throats of hens and pigeons are not the same as in human diphtheria. Friedberger states that he has never known a single instance of the disease being communicated from one bird to another. Ostertag considers this as strong evidence against the flesh being injurious to man. Klein isolated from the deeper parts of the deposits a number of micro-organisms, including bacilli of about the same size as those of fowl cholera, but differing from the latter in forming a thick yellow growth on potato.

Actinomycosis.—This disease is of frequent occurrence among cattle and swine, being, as a rule, sporadic. Horses are also occasionally infected, and the disease is not uncommon in the human subject. In cattle, firm tumours are formed, especially on the tongue, which is enlarged. In the lungs, where they are also sometimes found, they have considerable resemblance to tubercles.

Actinomyces.—This was first discovered by Langenbeck in 1845, and described by J. Israel in 1878. Within each of the tumours minute yellowish granules are met with, which, when examined under the microscope, are seen to have a characteristic radiating structure composed of club-shaped bodies. They can be cultivated on agar-agar and on blood serum, forming moist white colonies, some of which in the course of a few days turn yellow or light brown. The cultivations are composed of filaments and never the club-shaped bodies. An efflorescence appears on the surface of old cultivations, and masses of cocci can be observed.

Klein * considers that this micro-organism occurs naturally on grain, and is introduced into the animal's system through a wound or abrasion.

On injection of pure cultivations the club-structures are produced in the inoculated animal.

The Flesh of Infected Animals.—When the disease is local Ostertag denies that the flesh, other than that of infected parts, is dangerous; but when the disease is general he considers that none of the flesh should be sold. There is no evidence that man can be directly infected with the disease from animals, though this is not impossible.

Bothriomycosis.†—This is a chronic disease characterised by the formation of tumours in the connective tissues. It has hitherto only been found in the horse, and on one occasion in the ox. In the intermuscular connective tissue of the infected animal knots of varying size may be observed composed of a yellowish-brown substance, and occasionally containing yellowish-white granules, which under the microscope are seen to be grape-like zooglœal conglomerations of from 5 to 100 μ in diameter. They can be stained with gentian violet and methylene blue. The micro-organism causing the disease is the *bothriomyces* of Bollinger (1869).

Rabe found that pure cultivations of the fungus were pathogenic to guinea-pigs, and on inoculation produced œdema in sheep and goats. There is no evidence as to whether infected flesh is injurious to man.

The Muscle Ray-Fungus.†—In 1884 Dunckler in examining sections of meat for trichinæ noticed the presence of certain dark bodies, which, when magnified, were found to have a radiating structure. He considered it to be a variety of actinomycosis. Johne and others opposed this view.

In certain cases the muscular fibres lose their cross striation. According to Hertwig it is most frequently met with in the muscles of the abdomen and between the ribs. Ostertag states that in Berlin it is customary for the meat inspectors to condemn altogether only those swine which are so much infected that the muscle has a greyish-red appearance and is watery. The frequently occurring cases of slight infection are ignored. No instance of ill-effects of such flesh on man has been recorded (*cf.* p. 259).

Pathogenic Bacteria in Shell-Fish.—There appears to be but little doubt that numerous cases of enteric fever have been produced through eating oysters and other shell-fish taken from an infected source. Herdmann and Boyce ‡ have recently investigated the nature of the bacteria occurring in oysters. They have not found the bacillus typhosus in any oysters obtained directly from the sea or in the market, although they have proved that when oysters are inoculated with that bacillus the micro-organisms are recoverable up to the tenth day.

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* *Micro-organisms and Disease*, p. 490.

† Ostertag, *loc. cit.*, p. 431.

‡ *Proc. Royal Soc.*, 1899, pp. 239–241.

According to their experience the typhoid bacillus does not increase in the tissues of the oyster, but perishes in its intestines. Sea water also appears to be inimical to its development.

Bacilli allied to the *B. coli communis* were frequently found by them in shell-fish sold in the towns, and especially in oysters, but there was no evidence as to their occurrence in molluscs taken from pure water, and they may, therefore, possibly indicate sewage contamination. In no instances were any organisms giving all the reactions of *B. typhosus* isolated, although some of the bacilli resembled them in certain characteristics.

CHAPTER XIV.

THE EXTRACTION AND SEPARATION OF PTOMAINES.

Of the various methods which have been proposed for the separation and isolation of ptomaines, the following have been most generally used.

Brieger's (later) Method.—The finely-divided substance is extracted with water slightly acidified with hydrochloric acid, the extract evaporated on the water-bath, filtered, and concentrated to a syrup. This is taken up with boiling 90 per cent. alcohol, the liquid filtered, cooled, and the filtrate treated with an excess of an alcoholic solution of mercuric chloride. The precipitate is collected after twenty-four hours, washed, suspended in water, and the mercury removed by means of hydrogen sulphide. The filtered liquid now contains the ptomaines in the form of their hydrochlorides.

Gautier's objections to this method are, that some ptomaines are not precipitated by mercuric chloride, and that not all the hydrochlorides are soluble, even in hot water.

Pouchet's Method.—The slightly acid aqueous extract is neutralised, heated to coagulate albuminous substances, and filtered. The bases in the filtrate are precipitated by tannin, and the tannates collected, washed, and treated with lead hydroxide in excess, in the presence of alcohol. The filtrate, on evaporation, leaves a syrup which is taken up with water and dialysed. The bases pass through the membrane, and the water containing them is evaporated *in vacuo*, and extracted successively with ether, petroleum spirit, and chloroform.

Gautier[*] regards this method as incomplete, since tannin does not precipitate all the ptomaines, and many of those which are precipitated are not soluble in ether, petroleum spirit, or chloroform.

The Stas-Gautier Method.—Gautier has worked out the following modification of Stas's method, which he regards as the most generally applicable.

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[*] *Les Toxines*, p. 56.

The finely-divided substance is digested for twenty-four hours with water containing 0·5 per cent. of tartaric acid, after which the liquid is filtered and the last portions separated by pressure. If the substance to be examined is liquid or nearly liquid, it is rendered slightly acid with tartaric acid, and if oily it is shaken in a flask containing carbon dioxide, with an aqueous 0·25 per cent. solution of oxalic acid.

The slightly acid extract or acidified original liquid is heated for a moment at 100° C., to coagulate albuminous substances, and is then cooled and filtered. The filtrate is evaporated *in vacuo* at 40° C. to a syrup, which is *Extract A*.

The distillate will usually contain substances carried over with the water, such as phenols, indols, volatile fatty acids, ammonia, substituted ammonias, etc., with traces of volatile ptomaines. The latter are recovered by precipitating them with sulphuric acid in slight excess, and treating the sulphates with lime, which liberates the bases and removes the larger proportion of the ammonia they contain. The mixture of calcium sulphate and free bases is shaken with ether, and then with alcohol, and any calcium oxide which dissolves is precipitated with a trace of sulphuric acid, leaving the bases in solution.

The *Extract A* is extracted with ether, which removes fatty substances, lactic acids, the excess of acid added, etc., and is then treated with boiling alcohol, which gives

Solution B, and leaves *Residue C*.

The *Residue C*, which contains salts, extractives, xanthic bodies, acid amides, etc., is taken up with water and dialysed.

The part passing through is concentrated by evaporation, the bases it contains precipitated with lead acetate, the lead removed by means of hydrogen sulphide, the filtrate concentrated, and alcohol added. The substances which gradually deposit consist of oxygenated bases, such as leucine.

The *Solution B*, which contains the most important bases, with peptones, etc., is evaporated to a syrup, rendered alkaline with potassium bicarbonate, mixed with powdered glass, and extracted successively with ether, chloroform, and amyl alcohol.

The two first extracts on evaporation leave as a residue any alkaloidal substances which may be present. The amyl alcohol extract is shaken with water slightly acidified with sulphuric acid, which takes up the bases in solution. The liquid is boiled, and a hot solution of barium hydroxide added so long as a precipitate forms. The bases, which are left in the solution, can be separated into fixed and volatile bases by distillation, the distillate being received in acidulated water.

Dragendorff's Method.—The finely-divided substance is mixed with water containing a little sulphuric acid, digested for several hours at 50° C., and washed with water. The liquid is evaporated to a syrup, and digested for twenty-four hours with three or four times its volume of 95 per cent. alcohol. The separated substances are filtered off, the alcohol evaporated from the filtrate, and the aqueous residue shaken with benzene, which removes certain impurities.

The residue is rendered alkaline with ammonia, and again extracted with benzene, which this time removes certain liberated bases.

The liquid is then acidified and extracted with chloroform, again rendered alkaline with ammonia or sodium carbonate, and again extracted with the same solvent. In like manner, extractions are made with amyl alcohol, first from acid and then from alkaline solution. Finally, the bases are recovered from each of the several extracts and examined.

Gautier's objections to this method are that there is a danger of forming basic substances by the action of the sulphuric acid on certain organic substances (lecithins, etc.), and that some ptomaines are readily attacked by dilute mineral acids.

DESCRIPTION OF PTOMAINES INCLUDED IN GAUTIER'S SYSTEM OF CLASSIFICATION.

The following description of the characteristics of the different ptomaines is, in the main, compiled from the treatises of Brieger and of Gautier, who may be regarded as the greatest authorities on the subject:—

AMINES OF THE FATTY ACID SERIES.

I.—Monamines.

METHYLAMINES.

Trimethylamine. $(CH_3)_3N$.

This has been met with in herring pickle, ergot of rye, human urine, normal blood, in the products of yeast putrefaction, and in those of meat, cheese, etc.

It appears to be derived especially from lecithins, which, under the influence of hydrating agents, are converted into glycerophosphoric acid, fatty acids, and choline, $N(CH_3)_3(C_2H_4.OH)OH$. The latter disappears after some days of putrefaction, being converted into trimethylamine and other substances.

Properties.—At ordinary temperatures trimethylamine is a gas with a characteristic fish-like odour. It boils at

9·3° C., and does not solidify at −75° C. It is very soluble in water, and forms well-marked salts, of which the aurochloride forms yellow monoclinic prisms, and the platinochloride orange crystals.

ETHYLAMINES.

Ethylamine [C_2H_7N or $C_2H_5.NH_2$] has been found in the putrefactive products of flour and yeast. It is a strongly alkaline liquid (B. P. 18·7° C.) with an ammoniacal odour.

Diethylamine [$(C_2H_5)_2NH$] occurs in fish exposed for some days to the air, and in decomposing meat extract and sausages. It is a volatile imflammable liquid, which is very soluble in water. It can be separated from ethylamine by treating the mercurochlorides with acetic acid, in which the diethylamine salt is insoluble. It boils at 57·5° C.

Triethylamine [$(C_2H_5)_3N$] has been found accompanying ethylamine and diethylamine and bases such as neuridine and gadinene in the products of the putrefaction of peptones and of fish. It is a strongly alkaline, inflammable liquid boiling at 89° to 89·5° C. It is slightly soluble in water, and is precipitated from its solutions by mercuric salts, and by salts of copper, lead, iron, etc. The aurochloride soon darkens from its reduction to aurous chloride.

PROPYLAMINES [C_3H_9N]

have been found in decomposing cod liver and putrid gelatin. Their salts increase the secretion of the sweat glands.

Normal propylamine [$CH_3.CH_2.CH_2.NH_2$] is a mobile ammoniacal liquid (B. P. 78° to 82° C.) soluble in water. Its platinochloride forms monoclinic crystals.

Isopropylamine [$(CH_3)_2.CH.NH_2$] is a liquid with an ammoniacal odour. It boils at 32° C., and is soluble in water. Its platinochloride crystallises in orange plates.

BUTYLAMINES [$C_4H_9.NH_2$].

A butylamine was found by Gautier in the water in which cod livers had been kept. On injection it produced stupor in small doses, and muscular paralysis and convulsions in larger doses. Its salts increased the activity of the renal glands.

Normal butylamine is a liquid boiling at 76° C., and soluble in water. It reduces alkaline solutions of copper and silver. Its platinochloride crystallises in yellow plates which are fairly soluble in water.

AMYLAMINES $[C_5H_{11}.NH_2]$.

Several of the amines with this composition have been isolated. From cod-liver oil Gautier * extracted one which he found to form about two-thirds of the total bases in the oil. From its composition it appeared to be iso-amylamine $[(CH_3)_2.CH.CH_2.CH_2.NH_2]$. It was a colourless, mobile liquid with a disagreeable odour and a specific gravity at 0° C. of 0·797. It attracted carbon dioxide from the air, and formed a very soluble hydrochloride. When injected into a dog it produced convulsions, dilation of the pupil of the eye, and feeble respiration and pulse.

HEXYLAMINES $[C_6H_{13}.NH_2]$.

These have also been found in cod's liver and in putrefying yeast. They are less poisonous than the amylamines.

Normal hexylamine $[CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2]$ is a liquid boiling at 129° C. Its hydrochloride crystallises in laminæ, and its platinochloride in scales.

II.—**Diamines.**

ETHYLIDENEDIAMINE (?) $[C_2H_8N_2]$.

A ptomaine was extracted by Brieger from putrid fish, which at first he regarded as ethylenediamine $[NH_2CH_2.CH_2.NH_2]$, but which he subsequently found was not identical with synthetically prepared ethylenediamine. Its hydrochloride crystallises in long needles, which are very soluble. It is poisonous, producing, on injection, lethargy, dilation of the pupil of the eye, increased secretion of the glands of the eyes, nose, and mouth, and death after twenty-four hours.

TRIMETHYLENEDIAMINE (?) $[C_3H_{10}N_2]$.

Brieger † isolated from a cultivation of Koch's comma bacillus a base with the above empirical formula, which was possibly trimethylenediamine $[NH_2.CH_2.CH_2.CH_2.NH_2]$. It was accompanied by cadaverine and kreatinine, from which it was separated by the following method :—The total bases were precipitated with an alcoholic solution of mercuric chloride, the mercury removed from the precipitate, and the bases precipitated with sodium picrate. The picrates were treated with boiling absolute alcohol, which dissolved the cadaverine picrate. The residual bases were converted into platino-chlorides, of which that of kreatinine, being much more soluble, could be washed out.

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* *Les Toxines*, p. 76.

† *Untersuch. üb. Ptomaïne*, i. p. 45 and ii. p.

The trimethylenediamine (?) also gave a fairly insoluble precipitate with gold chloride. It was very poisonous, producing, on injection, muscular twitchings and convulsions.

PUTRESCINE or TETRAMETHYLENE-DIAMINE [$C_4H_{12}N_2$].

This base was found by Brieger * in the putrefactive products of flesh. It was accompanied by cadaverine and neuridine, and was abundant after the eleventh day. In putrefaction, neuridine appears to be formed first, and to be replaced by cadaverine and putrescine. It has also been found in cultivations of *B. coli communis*, and in decomposing solutions of gelatin.

Separation from Neuridine and Cadaverine.—The platino-chlorides of the three bases are treated with cold water, in which that of putrescine is but slightly soluble. It dissolves in hot water, but crystallises out before that of cadaverine. The free bases are obtained by treating the platino-chlorides with hydrogen sulphide, and distilling the hydrochlorides formed with sodium hydroxide.

Properties and Reactions.—Putrescine is a clear, mobile liquid with a characteristic odour resembling, to some extent, that of the pyridine bases. It absorbs carbon dioxide from the air with avidity, forming a crystalline carbonate with the same unpleasant smell. It boils at about 135° C. (Brieger), but when perfectly free from water at 158° C. (Udransky and Baumann). It melts at 27°–28° C., after being crystallised in a freezing mixture. It is only volatile with difficulty in a current of steam.

Brieger gives the following summary of the reactions of the free base:—

Phosphotungstic acid,	White precipitate, soluble in excess.	
Phosphomolybdic acid,	Yellow precipitate.	
Potassium mercury iodide,	Oily precipitate, afterwards becoming crystalline.	
Potassium bismuth iodide,	Do.	do.
Potassium cadmium iodide,	Do.	do.
Picric acid,	Yellow needles.	
Tannic acid,	Dirty, white precipitate.	

It forms crystalline salts with acids. The hydrochloride

$$[C_4H_{12}N_2.2HCl],$$

which forms long colourless transparent needles, is non-hygroscopic, very soluble in water, soluble with difficulty in dilute alcohol, and insoluble in absolute alcohol.

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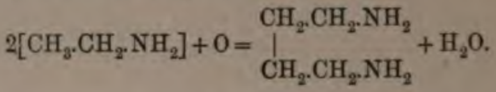
* *Weitere Untersuch. üb Ptomaïne*, ii. p. 42.

The platinochloride $[C_4H_{12}N_2.2HCl.PtCl_4]$, which is nearly insoluble in water, crystallises in hexagonal superposed plates. Putrescine itself is not poisonous, but its tetramethyl-derivative $[C_4H_8(CH_3)_4N_2]$ is exceedingly toxic, producing similar effects to those caused by neurine and muscarine.

Udransky and Baumann consider that putrescine is derived from the oxidation of ethylamine.



$$2[CH_3.CH_2.NH_2] + O = \begin{array}{l} CH_2.CH_2.NH_2 \\ | \\ CH_2.CH_2.NH_2 \end{array} + H_2O.$$

CADAVERINE OR PENTAMETHYLENE-DIAMINE
$[C_5H_{14}N_2]$ or $[NH_2.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2]$.

This base, to which Brieger assigned the formula $C_5H_{16}N_2$, has been proved by Ladenburg to contain two atoms of hydrogen less. It appears to develop between the third and fifteenth day of the putrefaction of flesh, and has been found in fresh pancreas extract. It is often associated with neuridine and putrescine, appearing before these disappear.

Separation from Putrescine and Neuridine.—Brieger precipitated the hydrochlorides with an alcoholic solution of mercuric chloride. The platinochlorides were prepared and fractionally crystallised, those of cadaverine and putrescine being but little soluble, while that of neuridine is much more soluble. On treating the more insoluble platinochlorides with hydrogen sulphide the hydrochlorides are formed, and on treating these with hot alcohol (96 per cent.) cadaverine hydrochloride is dissolved.

Properties and Reactions.—Cadaverine is a transparent, viscid liquid, which fumes in the air, and rapidly absorbs carbon dioxide, solidifying to a crystalline mass. It boils at 115° to 120° C. (Brieger), and at 175° to 178° C. when dehydrated. It has an oily penetrating odour, resembling that of piperidine.

The chief reactions of the free base are—

Phosphotungstic acid, . .	White precipitate, readily soluble in excess.
Phosphomolybdic acid, . .	Do. do. do.
Phosphoantimonic acid, .	White crystalline precipitate.
Potassium mercury iodide, .	Resinous precipitate.
Potassium cadmium iodide, .	Resinous precipitate, gradually becoming granular.
Potassium bismuth iodide, .	Brown precipitate.
Iodine in potassium iodide, .	Brown precipitate.
Picric acid, ...	Yellow needles.
Tannic acid, ...	White amorphous precipitate
Potassium ferricyanide and ferric chloride, . . .	Blue coloration.

The hydrochloride [$C_5H_{14}N_2.2HCl$] crystallises in deliquescent needles, which are soluble in water, and moderately soluble in alcohol. It gives yellow needles with picric acid, and brown needles with iodine in potassium iodide. With iron chloride and potassium ferricyanide it gives a faint blue colour.

The platinochloride [$C_5H_{14}N_2.2HCl.PtCl_4$] forms reddish-yellow prisms, which are moderately soluble in cold water.

On dry distillation cadaverine is decomposed into ammonium chloride and piperidine ($C_5H_{11}N$).

It has little or no effect in small doses, but when injected in large quantity into mice is poisonous, a *post-mortem* symptom being that the coagulation of the blood is retarded.

NEURIDINE [$C_5H_{14}N_2$].

This base, which has the same empirical formula as cadaverine, is formed during the first five or six days of the putrefactive decomposition of meat, fish, albumin or gelatin, and reaches its maximum on the eleventh or twelfth day. It is also found among the products of the cultivation of Eberth's bacillus.

*Brieger's Method of Isolating Neuridine.**—The finely-divided flesh was left to putrefy for five or six days. The mass was then extracted with hot water slightly acidified with hydrochloric acid, the extract filtered, concentrated on the water-bath to a syrup, and repeatedly extracted with alcohol. The alcoholic filtrate was precipitated with mercuric chloride, the precipitate decomposed with hydrogen sulphide, and the liquid filtered. The filtrate, on concentration on the water-bath, yielded long needle-shaped crystals of neuridine hydrochloride, which were purified by recrystallisation from small quantities of hot dilute alcohol. When choline was also present, its hydrochloride remained in the mother liquid. It could also be separated from neuridine hydrochloride by the readiness with which it dissolved in absolute alcohol.

Properties and Reactions.—Free neuridine is insoluble in absolute alcohol and ether, soluble with difficulty in amyl alcohol, but readily soluble in water. It is unstable, and its solution slowly decomposes, even when concentrated *in vacuo*. It gives white precipitates with mercuric chloride, and with normal and basic lead acetate. The hydrochloride [$C_5H_{14}N_2.2HCl$] is very soluble in water. It gives the following reactions.

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* *Untersuch. üb. Ptom.*, Pt. i. and ii., pp. 18 and 19.

Phosphotungstic acid, . . .	White amorphous precipitate. Soluble in excess.
Phosphomolybdic acid, . . .	White crystalline precipitate.
Phosphoantimonic acid, . .	White flocculent precipitate.
Picric acid, ...	Precipitate slowly appears. Soon changes to yellow needles.
Potassium bismuth iodide, .	Red amorphous precipitate.
Gold chloride, ...	Crystalline precipitate.

It gives no precipitate with mercuric chloride in aqueous solution, with tannic acid, with Fröhde's reagent, or with a mixture of potassium ferricyanide and ferric chloride.

The picrate $[C_5H_{14}N_2.2(C_6H_2(NO_2)_3OH)]$ is very soluble. The platinochloride $[C_5H_{14}N_2.2HCl.PtCl_4]$, which crystallises in needles, is soluble in water, and precipitated by alcohol.

Neuridine appears to be without physiological action. It occurs as a leucomaine in undecomposed animal tissues, in egg yolk, etc.

SAPRINE $C_5H_{14}N_2$ (Gautier), $C_5H_{16}N_2$ (Brieger).

This base, which appears to be isomeric with the two preceding ptomaines, was isolated by Brieger from decomposing human flesh.

Isolation and Separation from Cadaverine and Putrescine.—The hydrochlorides are dissolved in hot alcohol, in which that of putrescine is less soluble. The filtrate is slightly concentrated, and platinum chloride added. The cadaverine platinochloride crystallises out first in an almost pure state, and then, on continued concentration, the saprine platinochloride is gradually deposited.

Differences from Cadaverine.

	CADAVERINE.	SAPRINE.
Platinochloride, .	Soluble with difficulty in water. Crystallises in rhombs.	Readily soluble in water. Crystallises in parallel needles.
Hydrochloride, .	Gradually deliquesces on exposure to air.	Crystallises in broad needles, which do not deliquesce in the air.
Aurochloride, .	Crystallises in readily soluble needles.	Is not formed on adding gold chloride to the solution.

Properties and Reactions.—Saprine can be distilled unchanged in a current of steam. It has a faint odour of pyridine. It resembles cadaverine in most of its reactions, but forms with potassium bismuth iodide an amorphous instead of, like cadaverine, a crystalline precipitate. The pure base gives an intense blue coloration with ferric chloride and potassium ferricyanide. It is physiologically inactive.

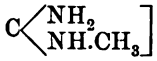
The base *Gerontine* has the same composition as saprine. It is a leucomaine found in the hepatic glands of a dog.

III.—Triamines and Tetramines of the Fatty Acid Series.

No ptomaines which can be classified under this head have, as yet, been described (Gautier).

GUANIDINES.

Various ptomaines with the constitution of guanidines have been described, such as methylguanidine, glycocyamidine and propylglycocyamine, but with the exception of methylguanidine they have only been found among the products of pathogenic bacteria, and not as putrefactive bases.



METHYLGUANIDINE [$C_2H_7N_3$ or $NH : C\begin{cases} NH_2 \\ NH.CH_3 \end{cases}$]

was isolated by Brieger from putrefied horseflesh, and by Bocklisch from decomposed beef extract. It has also been found in pure cultivations of various bacteria, such as those of mouse septicæmia.

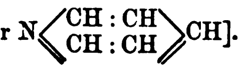
Properties.—The free base is crystalline, deliquescent and very alkaline. Treated with potassium hydroxide, it gives off ammonia and methylamine.

The hydrochloride [$C_2H_7N_3.HCl$] is insoluble in alcohol. The platinochloride [$(C_2H_7N_3HCl)_2.PtCl_4$] forms rhombic crystals, which only dissolve with difficulty in water and alcohol, but are readily soluble in ether. The picrate is a resin-like substance, which is only sparingly soluble.

Methylguanidine is very poisonous, and, on injection, produces muscular trembling, convulsions, dilation of the pupil of the eye, paralysis and death.

AROMATIC PTOMAINES NOT CONTAINING OXYGEN.

I.—Aromatic Monamines.

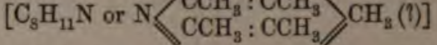


PYRIDINE [C_5H_5N, or $N\begin{cases} CH : CH \\ CH : CH \end{cases}CH$].

This has been found among the decomposition products of proteids. It is a colourless liquid with a characteristic odour.

It boils at about 116° C., and is miscible with water. It forms a pale blue precipitate with copper sulphate, which dissolves in an excess of the base.

COLLIDINE or TRIMETHYLPYRIDINE (?)



$$[C_8H_{11}N \text{ or } N \left\langle \begin{matrix} CCH_3 : CCH_3 \\ CCH_3 : CCH_3 \end{matrix} \right\rangle CH_3 \,(?)]$$

was separated by Œchsner and Coninck from putrid cuttle-fish. It is a yellow liquid with an acrid odour. It is slightly soluble in water, and readily soluble in alcohol, ether and acetone. Its density is 0·986, and its boiling-point 168° C.

The hydrochloride is crystalline, deliquescent, and very soluble.

The platinochloride $[(C_8H_{11}N.HCl)_2.PtCl_4]$ forms red crystals, which are readily soluble in hot water. It is very poisonous.

Nencki extracted from putrefied gelatin, a base with the same composition.

PARVOLINE $[C_9H_{13}N]$.

This poisonous pyridine base was discovered by Gautier and Étard in horseflesh which had been exposed for several months in hot weather.

It is an oily amber-coloured substance, boiling above 200° C. It is slightly soluble in water, and very soluble in alcohol, ether and chloroform. On exposure to the air it turns brown.

The platinochloride is precipitated as a crystalline mass, which turns brown on exposure to light and air. It does not dissolve readily.

CORINDINE $[C_{10}H_{15}N]$.

A base with this chemical composition was isolated by Œchsner, together with collidine from putrefied cuttlefish. It is a yellow, somewhat viscous liquid, which boils at about 230° C., and has a disagreeable odour. It is soluble in alcohol, ether, and acetone, but only slightly soluble in water. On exposure to the air it becomes a resinous mass.

The hydrochloride forms deliquescent needle-shaped crystals, which are very soluble. The platinochloride is a reddish powder, insoluble in water.

Corindine is poisonous, causing paralysis on injection.

DIHYDROCOLLIDINE $[C_8H_{13}N]$.

This base was discovered by Gautier and Étard in 1881 in putrefied meat and fish, where it was accompanied by some of the pyridine bases mentioned above. It is a nearly colourless, slightly oily liquid with a penetrating odour. It attracts carbon dioxide from the air, forming a crystalline carbonate. It boils at about 208° C., and has a density of 1·0296 at 0° C. The hydrochloride crystallises in fine needles, which are very soluble in alcohol and ether. The platinochloride forms pale yellow, curved crystals, which are not easily soluble.

On injection this ptomaine produces stupor, muscular paralysis, convulsions and death.

DIHYDROLUTIDINE [$C_7H_{11}N$].

This was found by Gautier and Margues in cod-liver oil. It is a colourless, oily liquid, strongly alkaline, and with a strong but not unpleasant odour. It absorbs carbon dioxide from the air. It boils at 199° C., and is slightly soluble in water.

The hydrochloride is crystalline, and very soluble, but not deliquescent. It is partially dissociated at 100° C.

The platinochloride crystallises in yellow plates, and occasionally in fine needles.

This ptomaine is very poisonous, producing, on injection, muscular trembling, partial paralysis, and asphyxia.

BASE [$C_{32}H_{31}N$].

A ptomaine corresponding in composition with this formula was extracted by Delezinier from putrefied flesh. It is an oily, nearly colourless liquid, readily oxidised on exposure to the atmosphere. It resembles the alkaloid veratrine in its physiological effects.

II.—Aromatic Diamines.

MERLUSINE [$C_8H_{12}N_2$].

Found by Gautier in cod's liver and in the water in which it had been kept. It is an oily, alkaline liquid, somewhat soluble in water and very soluble in ether. It forms a crystalline acetate and platinochloride.

III.—Aromatic Triamines.

MORRHUINE [$C_{19}H_{27}N_3$] and HOMO-MORRHUINE [$C_{20}H_{29}N_3$].

These bases were found by Gautier in the part of the bases of cod-liver oil which were soluble in ether.

Morrhuine is a viscous yellow oil, with a sweet odour and alkaline reaction. It precipitates copper from the solutions of its salts, but the precipitate does not dissolve on adding an excess of the base. The hydrochloride, which is very deliquescent, crystallises in stellate groups. The platino-chloride forms microscopic needles.

Homo-morrhuine closely resembles morrhuine in its properties. It is a viscous base of a yellow colour, and forms well-defined crystalline salts. The platinochloride $[(C_{20}H_{29}N_3HCl)_2.PtCl_4]$ is a yellow salt readily soluble in hot water.

According to Gautier these two bases constitute more than a third of the total bases of cod-liver oil. They are but little, if at all, poisonous, but have marked diuretic and stimulating properties, and Gautier considers that part of the physiological action of cod-liver oil is to be attributed to their presence.

IV.—**Aromatic Tetramines.**

NICOMORRHUINE $[C_{20}H_{28}N_4]$.

Gautier found this base accompanying morrhuine and homo-morrhuine in cod liver which had been exposed to 'fermentation' before the extraction of the oil. It has the same percentage composition as nicotine $C_{10}H_{14}N_2$, but from the composition of its platinochloride Gautier doubled the formula.

Properties.—It is a viscous oil with a faint odour of tobacco. It is slightly soluble in water, and has a density of about 1. Its hydrochloride crystallises in nacreous plates, which are very soluble in water, but not very deliquescent.

The platinochloride $[C_{20}H_{28}N_4.2HCl.PtCl_4]$ forms a flocculent brick-red precipitate insoluble in water.

This ptomaine is somewhat poisonous.

ASELLINE $[C_{25}H_{32}N_4]$.

This is another base isolated by Gautier from cod-liver oil. On separating the basic substances of this oil by distillation, a brown residue is obtained, from which the morrhuines and nicomorrhuine can be extracted with ether. The insoluble portion contains a large proportion of a base with the above formula.

Properties and Reactions.—The free base is an amorphous, greyish mass emitting an aromatic odour on warming. Its density is about 1·05. It is slightly soluble in water and ether, very soluble in alcohol. It combines with acids to form crystalline salts.

Its reactions may be summarised thus:—

Sulphuric acid, . .	Rose coloration, changing to brown.
Hydrochloric acid, .	Small crystals arranged in an X-form. Bitter taste.
Gold chloride, . . .	Gives a salt which is decomposed on treatment with boiling water.
Mercuric chloride, .	White precipitate, soluble on heating, and depositing as a crystalline mass on cooling.
Platinum chloride, .	Yellow or orange precipitate, dissolving without alteration on boiling.

Gautier states that aselline composes about one-fifteenth of the total bases of cod-liver oil. Physiologically, it produces stupor and respiratory troubles in small doses, and convulsions and death when injected in larger quantity.

SCOMBRINE [$C_{17}H_{38}N_4$]

was found by Gautier and Étard in the mother-liquid left on filtering off hydrocollidine platinochloride, obtained during the putrefaction of fish, especially the mackerel. The platinochloride ($C_{17}H_{38}N_4.2HCl).PtCl_4$ crystallises in yellow needles.

PTOMAINES CONTAINING OXYGEN OR SULPHUR.

Gautier subdivides these into three groups—(i.) Neurinic bases; (ii.) Aromatic bases containing oxygen; (iii.) Amides or acid amides; and (iv.) Carbopyridic acids.

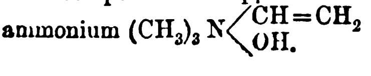
I.—Neurinic Bases.

The ptomaines allied to neurine which have been found in the products of putrefaction are choline, muscarine, mytilotoxine (in poisonous mussels), a base with the composition $C_7H_{17}NO_2$, betaine, gadinene, and mydatoxine.

NEURINE [$C_5H_{13}NO$].

This base was discovered as a ptomaine by Brieger, who found it in the putrefaction products of flesh towards the fifth or sixth day, together with other bases, including neuridine and muscarine. It appears to be formed in putrefaction from choline [$C_5H_{15}NO_2$] by the loss of a molecule of water. Choline, itself, is a decomposition product of lecithins, and is very unstable, being converted into neurine by heat or by the action of acids or alkalies. When the lecithins of the brain are acted upon by acids or bases, both choline and neurine are formed. Liebreich (1869) found that impure cerebral lecithin yielded neurine when heated for twenty-four hours with barium hydroxide.

In composition it appears to be a hydroxide of trimethyl-vinyl-ammonium



$$(CH_3)_3N\begin{cases}CH=CH_2\\OH.\end{cases}$$

Properties and Reactions.—The free base is a syrupy liquid, with a very alkaline reaction, and giving off fumes on contact with hydrochloric acid. It is soluble in water, and is only removed in small proportion from the solution by ether, petroleum spirit, chloroform, and amyl alcohol. When concentrated solutions are boiled a small quantity of trimethylamine is evolved.

Neurine chloride $[C_5H_{12}NCl$ or $C_5H_{13}NO + HCl - H_2O]$ crystallises in fine needles, which are very hygroscopic.

Brieger gives the following table of its reactions with different reagents :—

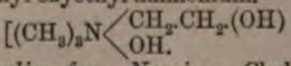
Phosphomolybdic acid, . .	White crystalline precipitate, soluble in excess.
Phosphotungstic acid, . .	*Nil*.
Phosphoantimonic acid, .	Voluminous white precipitate.
Potassium mercury iodide, .	Voluminous yellowish-white precipitate.
Potassium bismuth iodide, .	Amorphous red precipitate.
Potassium cadmium iodide,	White precipitate.
Iodine in potassium iodide,	Amorphous brown precipitate.
Tannin, ...	Voluminous dirty-white precipitate.
Mercuric chloride, . . .	White granular precipitate.

Neurine is extremely poisonous, the symptoms varying somewhat with different animals. Speaking generally, it produces salivation, and ejaculation of other secretions. The pupils of the eyes are often contracted. In fatal doses there are sudden convulsions, and death rapidly ensues.

Atropine has been found to be an active antidote, but neurine does not appear to be antidote for atropine.

CHOLINE $[C_5H_{15}NO_2]$.

This base was first found by Strecker in bile, and also occurs as a normal constituent in the blood, muscles and glands of animals, and in extracts of various vegetable substances (*e.g.*, certain fungi). It is formed, together with neurine, in the products of the decomposition of lecithins, by acids or alkalies. Wurtz prepared it synthetically from trimethylamine and the monochlorhydrin of glycol. According to Baeyer it is the hydroxide of trimethyl-oxyethyl-ammonium.



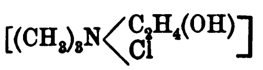
$$[(CH_3)_3N\begin{cases}CH_2.CH_2.(OH)\\OH.\end{cases}$$

Separation of Choline from Neurine.—Choline chloride is not precipitated by tannic acid, whilst neurine chloride is precipitated. On the other hand, phosphotungstic acid gives a precipitate with choline chloride, but not with neurine chloride.

Separation of Choline from Neuridine.—On the addition of picric acid to the solution of the mixed chlorides, neuridine picrate is immediately precipitated, while choline picrate remains in solution, and is only precipitated on concentrating the filtrate.

The aurochloride of neuridine is also much less soluble in water than that of choline.

Properties and Reactions.—The chloride of choline



$$\left[(CH_3)_3N\begin{cases}C_2H_4(OH)\\Cl\end{cases}\right]$$

forms very deliquescent needles, which are soluble in absolute alcohol. It gives the following reactions (Brieger):—

Phosphotungstic acid, . .	White precipitate, insoluble in water. Becomes crystalline on standing.
Phosphomolybdic acid, .	Voluminous precipitate.
Phosphoantimonic acid, .	White caseous precipitate.
Potassium mercury iodide.	Yellow crystalline precipitate.
Potassium bismuth iodide,	Red amorphous precipitate.
Iodine in potassium iodide,	Brown granular precipitate.
Mercuric chloride, . . .	White granular precipitate.
Tannin, ...	*Nil.*

Free choline is a syrupy liquid, very soluble in water. In a 2 per cent. solution it dissolves fibrin and coagulates albumin. When mixed with a large amount of water it is gradually transformed into neurine. Oxidising agents, such as dilute nitric acid, convert it into oxycholine or muscarine [$C_5H_{15}NO_3$], betaine [$C_5H_{11}NO_2$], and oxyneurine [$C_5H_{13}NO_3$].

The platinochloride [$((CH_3)_3)C_2H_4.OH(NCl_2)_2.PtCl_4$] is trimorphic in form, crystallising in plates, octahedra, or prisms. It invariably contains more or less water, which it does not lose at 110° C. It melts at 232° to 240° C.

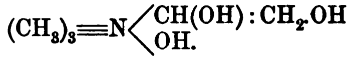
The aurochloride forms yellow needles, which dissolve with difficulty in water.

The mercurochloride [$C_5H_{14}NOCl.6HgCl_2$] is much less soluble than the corresponding salts of putrescine and cadaverine, a property which can be used to separate choline from these and certain other ptomaines. The alkaline extract of the bases is slightly acidified with hydrochloric acid, the liquid filtered, and, after neutralisation of the excess of acid, evaporated *in vacuo*. The dry residue is taken up with alcohol, and an alcoholic solution of mercuric chloride added to the liquid. The precipitate, when recrystallised once or twice, is obtained in crystalline needles, which, when decomposed by hydrogen sulphide, give choline chloride.

Choline resembles neurine in its physiological effects, but is much weaker in its action. Atropine is an antidote.

MUSCARINE [$C_5H_{15}NO_3$]

was found by Brieger associated with ethylidenediamine (?), neuridine, gadinene, and trimethylamine in putrid fish. It has been isolated from poisonous mushrooms, and is formed artificially by the oxidation of choline. In composition it agrees with the formula



$$(CH_3)_3{\equiv}N\begin{matrix}\diagup CH(OH):CH_2.OH\\ \diagdown OH.\end{matrix}$$

Brieger's Method of Separating Muscarine.—The alcoholic extract of the putrefaction products is precipitated with mercuric chloride, in order to separate the choline and neurine. The filtrate is treated with hydrogen sulphide to remove the mercury, and filtered. The filtrate is concentrated, after neutralisation with sodium hydroxide, and the syrupy residue taken up in alcohol and mixed with an excess of platinum chloride. The platinochloride of neuridine crystallises out first, and is filtered off. On concentrating the filtrate, the platinochloride of ethylidene diamine (?) crystallises out, and on further evaporation, that of muscarine. This third precipitate, on treatment with hydrogen sulphide, gives the hydrochloride, which is converted into the sulphate by treatment with silver sulphate, and the latter, when decomposed with barium hydroxide, gives the free base.

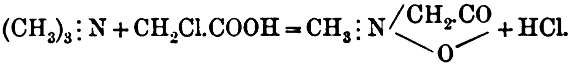
Properties and Reactions.—Muscarine forms colourless, deliquescent crystals. It is extremely alkaline, and absorbs carbon dioxide from the air.

The platinochloride. $[(C_5H_{14}NO_2Cl)_2.PtCl_4.2H_2O]$ crystallises in well-defined octahedra, which are sparingly soluble in water. The aurochloride forms needle-shaped crystals, which are nearly insoluble.

Muscarine is very poisonous, producing, even in small doses, salivation, contraction of the pupils of the eyes, diarrhœa, convulsions, and death. The action of atropine is antagonistic.

BETAINE $[C_5H_{11}NO_2]$.

This base is formed, together with muscarine, in the artificial oxidation of choline. It was found by Brieger in large quantity in both wholesome and poisonous mussels. It can be prepared synthetically by treating glycocoll with methyl iodide, dissolved in methyl alcohol, in the presence of potassium hydroxide; also by the action of trimethylamine on chloracetic acid



$$(CH_3)_3:N+CH_2Cl.COOH=CH_3:N\begin{matrix}\diagup CH_2.CO\\ \diagdown\ O\ \diagup\end{matrix}+HCl.$$

Properties and Reactions.—The free base $[C_5H_{11}NO_2]$ forms brilliant crystals, which are deliquescent and dehydrated at 100° C.

The hydrochloride crystallises in monoclinic plates, insoluble in absolute alcohol.

The platinochloride $[(C_5H_{11}NO_2.HCl)_2.PtCl_4.+4H_2O]$ is soluble in water.

The mercurochloride is very soluble. The aurochloride dissolves with difficulty in cold water, but can be crystallised in plates from its solution in boiling water.

Betaine causes a slow reduction in a solution of potassium ferricyanide with ferric chloride.

Physiologically it appears to have no definite action on the system.

HOMO-PIPERIDINIC ACID, OR δ-AMIDO-VALERIC ACID [$C_5H_{11}NO_2$].

This base, isomeric with betaine, was isolated by E. and H. Salkowski from the products of the putrefaction of meat fibrin.

It crystallises in needles, which melt at 156° C., and dissolve readily in water, but only with difficulty in alcohol. It is not precipitated by copper acetate or by ammoniacal silver nitrate. According to Gabriel and Aschau it is identical with δ-amido valeric acid synthetically prepared. The hydrochloride is crystalline, and very soluble in water and concentrated alcohol.

It is non-poisonous. (*Cf.* p. 321.)

MYTILOTOXINE [$C_6H_{15}NO_2$].

This base was isolated by Brieger from poisonous mussels in the following manner:—The flesh was extracted with boiling water containing a trace of hydrochloric acid. The extract was evaporated to a syrup, exhausted with alcohol, and the filtered solution treated with lead acetate to remove mucilaginous substances. The filtrate from this precipitate was evaporated to dryness, the residue taken up with alcohol, the lead removed by treatment with hydrogen sulphide, the alcohol evaporated, and the residue dissolved in water and decolorised with animal charcoal. The filtered solution was neutralised with sodium carbonate, then acidified with nitric acid and precipitated with phosphomolybdic acid. The precipitate was decomposed by heating it with neutral (normal) lead acetate, the liquid filtered, and after removal of the lead, and addition of hydrochloric acid, evaporated to dryness. The residue was taken up with absolute alcohol, which left a little betaine undissolved, and precipitated with alcoholic mercuric chloride. The mercurochloride was purified by recrystallisation from boiling water, and finally converted into mytilotoxine hydrochloride by treatment with hydrogen sulphide.

Its composition is doubtful, but it is possibly a methyl derivative of betaine.

The hydrochloride crystallises in tetrahedra. The aurochloride melts at 182° C.

With regard to the physiological properties of mytilotoxine, see under 'Mussel Poisoning,' p. 221.

MYDATOXINE [$C_6H_{13}NO_2$].

This base was isolated by Brieger from horseflesh which had been left to putrefy for nine to fifteen months. It was accompanied by cadaverine and putrescine, and by a base with the formula $C_7H_{17}NO_2$.

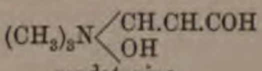
Brieger's Method of Isolating Mydatoxine.—The bases were precipitated as mercurochlorides, and the precipitate crystallised from boiling water. The mercurochloride of cadaverine separated first, and was filtered off. The filtrate was treated with hydrogen sulphide to remove mercury, filtered and evaporated, and the residue taken up with absolute alcohol, which left undissolved the hydrochloride of putrescine. The alcohol was evaporated and the mydatoxine precipitated as aurochloride, which was converted into hydrochloride by means of hydrogen sulphide, and this, when left in contact with silver oxide, gave the free base.

Properties and Reactions.—As thus obtained, mydatoxine is an alkaline, viscous liquid, which solidifies in plates on evaporation *in vacuo*. It is insoluble in alcohol and ether, and non-volatile.

Its reactions are:—

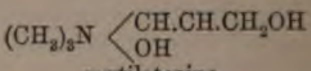
Hydrochloric acid, . . .	Deliquescent salt.
Platinum chloride, . . .	Small *lamellæ*, soluble in water. Melts about 193° C.
Gold chloride, ...	Give precipitates soluble in boiling water.
Mercuric chloride, . . .	
Potassium ferricyanide and ferric salts, ...	Rapid reduction takes place.

The relation between mydatoxine and mytilotoxine may be expressed as in the formulæ:—



$$(CH_3)_3N\Big\langle\begin{matrix}CH.CH.COH\\OH\end{matrix}$$

mydatoxine.



$$(CH_3)_3N\Big\langle\begin{matrix}CH.CH.CH_2OH\\OH\end{matrix}$$

mytilotoxine.

Mydatoxine is poisonous in large doses, causing diarrhœa and convulsions.

GADINENE [$C_7H_{17}NO_2$] and METHYL-GADINENE [$C_8H_{19}NO_2$].

These bases, which appear to be homologues of mytilotoxine, were extracted by Brieger from putrefied fish, especially cod. They were accompanied by muscarine and ethylidenediamine (?).

The more soluble platinochlorides were concentrated in the mother liquid, and after removing the muscarine salt the gadinene platinochloride was obtained in yellow platelets, which, on treatment with hydrogen sulphide, yielded the hydrochloride.

Properties and Reactions of Gadinene.—The platinochloride, when once deposited, can only be redissolved with difficulty. It melts at 214° C. The hydrochloride forms large, colourless needles, which are very soluble in water, but insoluble in alcohol.

Auric chloride, . . .	No precipitate. The auro-chloride is apparently not formed.
Picric acid, ...	Crystalline precipitates, only sparingly soluble.
Phosphotungstic acid, .	
Phosphomolybdic acid, .	

Gadinene has not marked poisonous properties. Gautier states that it is isomeric with typhotoxine.

Methyl-gadinene, which has also been found associated with mydatoxine in decomposed horseflesh, produces symptoms of tetanus when injected in sufficient quantity.

MYDALEINE.

This base was found by Brieger in human flesh after seven or eight days of putrefaction, being accompanied by neuridine, choline, cadaverine, putrescine, and saprine. It increases in quantity up to the twenty-fourth day of decomposition.

From Brieger's incomplete analysis of the platinochloride it appears to be a diamine, containing 4 or 5 atoms of carbon.

Since its mercurochloride was only insoluble in the strongest alcohol, it was impossible to completely separate it by the usual method. Advantage was therefore taken of the greater solubility of its platinochloride.

The hydrochloride crystallises with difficulty, and readily decomposes in the air. It gives the subjoined reactions:—

Platinum chloride, ...	Microscopic needles.
Gold chloride, ...	Oily drops.
Phosphomolybdic acid, ...	Yellow morphous precipitate.
Phosphotungstic acid, ...	White precipitate, soluble in excess.
Potassium mercury iodide, . . .	Oily yellow drops.
Potassium bismuth iodide, . . .	Dirty-brown oil.
Iodine in potassium iodide, . .	
Picric acid, ...	Yellow oil.
Potassium ferricyanide and iron chloride, ...	Immediate intense blue coloration.

Mydaleine is poisonous, producing dilation of the pupils of the eyes, salivation and tears, fever, diarrhœa, and paralysis.

BASE [$C_7H_{17}NO_2$].

This ptomaine, isomeric with gadinene and typhotoxine, was found by Brieger in putrid flesh, in association with mydatoxine.

Its hydrochloride forms fine needles, insoluble in strong alcohol. The auro-chloride [$(C_7H_{17}NO_2HCl).AuCl_3$] is dimorphous, and melts at 176° C. It gives a precipitate with copper acetate in the cold.

It does not appear to be an amido acid, since, although it has an acid reaction, it does not give a red coloration with ferric chloride (Hoffmeister's reaction).

It is poisonous, causing, on injection, first contraction and then dilation of the pupils, salivation, convulsive trembling, and death.

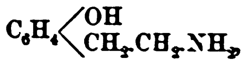
II.—Aromatic Ptomaines containing Oxygen.

TYROSAMINES [C_7H_9NO; $C_8H_{11}NO$; $C_9H_{13}NO$].

Gautier isolated these bases from cod's liver which had been kept in barrels, together with nicomorrhuine, amylamine, etc.

Gautier's Method of Separation.—The bases produced during the 'fermentation' were treated with ether, and the insoluble portion digested with amyl alcohol. A current of carbon dioxide was passed through the solution, by which potassium carbonate and certain bases were precipitated, while the tyrosamines and other bases remained in solution. The amyl alcohol was removed by washing with very dilute sulphuric acid, the acid precipitated with barium hydroxide, and the filtrate concentrated to a syrup, and boiled after the addition of water. The crystals, which had precipitated by the following day, were dissolved in water, and separated by fractional crystallisation into the two bases, C_7H_9NO and $C_8H_{11}NO_2$, while the third, $C_9H_{13}NO_2$, was obtained from the mother liquid.

The most abundant was the base $C_8H_{11}NO$, which Gautier regarded as paroxyphenyl-ethylamine



$$C_6H_4 \left\langle \begin{array}{l} OH \\ CH_2.CH_2.NH_2, \end{array} \right.$$

apparently derived from tyrosine by the loss of carbon dioxide.

It dissolves in 90 to 100 parts of water at 15° C., and forms better tasting salts.

The hydrochloride [$C_8H_{11}NO.HCl$] crystallises in plates and needles. The platinochloride is fairly soluble.

The salts of these bases are not poisonous.

MYDINE [$C_8H_{11}NO$].

This ptomaine, isomeric with one of Gautier's tyrosamines, was found by Brieger in putrid flesh, and in cultivations of the *Bacillus typhosus*. It is an alkaline base with strong reducing properties. It decomposes on distillation. Its hydrochloride is crystalline, and reduces ferricyanide mixed with a ferric salt. Its platinochloride is very soluble. The picrate melts at 195° C. It is not poisonous.

MORRHUAMINE [$C_{14}H_{20}N_2O_2$].

Another base isolated by Gautier from 'fermented' cod's liver. It was one of the substances precipitated by carbon dioxide in the separation of the tyrosamines.

It is a very hygroscopic and very alkaline base, partially volatilising at 110° C. It forms a crystalline hydrochloride and a soluble platinochloride.

LYSINE [$C_6H_{14}N_2O_2$].

This base is also one of the final products of pancreatic digestion. (See p. 184.)

POUCHET'S BASES [$C_5H_{12}N_2O_4$ and $C_7H_{18}N_2O_6$].

These were extracted from decomposed meat. They were precipitated with tannin, and the tannates decomposed, taken up with alcohol, and dialysed. The dialysed part yielded two platinochlorides, which could be precipitated by a mixture of alcohol and ether. One of these [$(C_7H_{18}N_2O_6.HCl)_2PtCl_4$] was insoluble in concentrated alcohol, but the other was fairly soluble, and could be separated as a yellow powder on the addition of ether.

The ptomaine $C_7H_{18}N_2O_6$ forms microscopic prisms turning brown in the light, while the ptomaine $C_5H_{12}N_2O_4$ crystallises in needles and is more stable.

Both bases are poisonous, producing stupor and paralysis.

GUARESCHI'S BASE [$C_{14}H_{12}N_2O_4$].

This base was extracted by Guareschi from fibrin which had been left to putrefy for several months.

It crystallises in plates, which melt at 248–250° C., and are soluble in water, forming a neutral or slightly acid solution.

It gives various ptomaine reactions, and appears to be the acid amide, $C_{12}H_6(COOH)_2.(NH_2)_2$.

LEPIERRE'S BASE [$C_{16}H_{28}N_2O_4$].

This ptomaine was found, in small quantity, in a cheese of goat's milk which had produced symptoms of poisoning.

It is a crystalline, inodorous, slightly acid base, and is soluble in alcohol. The hydrochloride crystallises in large needles, which are very soluble. The platinochloride and aurochloride are crystalline salts.

It is slightly poisonous, causing diarrhœa and digestive disturbances.

TYROTOXINE or TYROTOXICON (DIAZOBENZENE) [$C_6H_5N_2.OH$].

This ptomaine was separated by Vaughan in 1883 from cheese which had caused illness. Sixteen kilogrammes of the cheese yielded only about one gramme of the ptomaine. It has since been found in ice-cream.

The cheese was extracted with acidified water, and the extract made alkaline with potassium hydroxide and extracted with ether. The ethereal extract was evaporated, the residue taken up with water, the residue from this solution again extracted with ether, which, on evaporation *in vacuo*, left microscopic needles.

Tyrotoxicon decomposes when heated to 90° C. in the presence of water. Its aqueous solution produced the same symptoms as the poisonous cheese. It does not give all the ordinary alkaloid reactions, although it forms a platinochloride and gives the Prussian-blue reaction.

On adding two drops of sulphuric acid to a few drops of a concentrated solution of phenol containing a trace of tyrotoxicon, an orange coloration is obtained. But this reaction is not conclusive, since it may also be given by nitrates, nitrites and by butyric acid.

III.—**Amides or Amido Acids.**

(*a.*) *Amido Acids of Fatty Acid Series.*

GLYCOCOLL or AMIDO-ACETIC ACID [$C_2H_3(NH_2)O$].

This has been found among the products of the putrefaction of various kinds of flesh. It is also one of the final derivatives of the pancreatic digestion of gelatin.

It forms crystalline salts with mineral acids, such as the hydrochloride $(C_2H_8NO_2)_2.HCl$.

Physiologically it is inoffensive.

BUTALANINE, or AMIDO-VALERIC ACID [$C_5H_{11}NO_2$ or $(NH_2)C_4H_8.COOH$]

accompanies leucine in the products of pancreatic digestion. Its hydrochloride is insoluble in ether, but very soluble in water. It is not precipitated by platinum chloride. It is non-poisonous (see p. 184).

δ-AMIDO-VALERIC ACID [$NH_2.CH_2.CH_2.CH_2,CH_2.COOH$].

This homologue of butalanine was isolated by E. and H. Salkowski from the products of the bacterial decomposition of albumin. It is a crystalline body, fairly soluble in water, slightly soluble in alcohol, and insoluble in ether. It melts at 156° C.

Its hydrochloride [$C_5H_{11}NO_2.HCl$] forms stellar crystals, which are non-deliquescent. The platinochloride is yellow and crystalline (*cf.* p. 315).

LEUCINE, or AMIDO-CAPROIC ACID [$C_6H_{13}NO_2$ or $NH_2.C_5H_{10}.COOH$].

This is a common constituent of putrefactive products, and is also produced in gastric and intestinal digestion.

Its hydrochloride is not precipitated by phosphotungstic acid or platinum chloride (see pp. 183 and 185).

AMIDO-STEARIC ACID [$C_{18}H_{35}(NH_2)O_2$].

This was found by Gautier and Étard in putrefied flesh.

The free acid is insoluble in water, but very soluble in hot alcohol. It crystallises in needles which melt at 63° C. When heated at 140° C. it loses water, and is apparently converted into its anhydride $C_{18}H_{35}NO$.

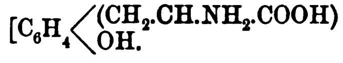
OTHER LEUCINES and LEUCEINES

are invariably found in the putrefaction products of meat or fish, such as more complex compounds of the general formula

$$C_nH_{2n-1}NO_2.$$

(*b*) *Amido Acids of Aromatic Series.*

TYROSINE [$C_9H_{11}NO_2$] or *p*-HYDOXYPHENYL-α-AMIDO PROPIONIC ACID



$$[C_6H_4\left\langle\begin{matrix}(CH_2.CH.NH_2.COOH)\\ OH.\end{matrix}\right.$$

This is invariably present in putrefactive products, and accompanies leucine as a normal constituent of the pancreas, liver and blood.

The hydrochloride is crystalline, and dissociated on contact with water. The platinochloride is very soluble and deliquescent.

PHENYL-AMIDO-PROPIONIC ACID [$C_6H_5.CH_2.CH.(NH_2)COOH$]

was found by Nencki in the products of the action of anaërobic bacteria on fibrin.

PTOMAINE [$C_{14}H_{20}N_2O_4$].

Guareschi isolated a substance with this formula from putrefied fibrin.

It forms crystalline lamellæ, soluble in water and alcohol, and melting at 247° to 250°.

The platinochloride forms rosette-shaped crystals. The hydrochloride is precipitated by phosphomolybdic acid (yellow mass), and by picric acid (reddish-yellow precipitate). Gold chloride gives a precipitate which is instantly reduced. It gives the Prussian blue reaction.

Guareschi considered this compound to be an amido acid, but Gautier doubts this conclusion, since it gives precipitates with phosphomolybdic acid and with Bouchardat's reagent.

IV.—Carbopyridic Acids.

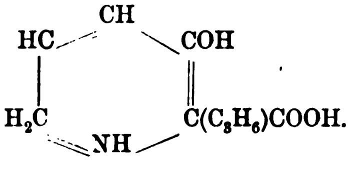
MORRHUIC ACID [$C_9H_{13}NO_3$].

This was isolated by Gautier from cod-liver oil. It is a feeble acid, which is insoluble in ether, and forms salts with alkalies.

Its platinochloride crystallises in soluble prisms. The aurochloride is amorphous.

Physiologically it acts as a stimulant to the appetite, and is non-poisonous.

Gautier considers that its probable constitution is



PATHOLOGICAL PTOMAINES.

In addition to the compounds described in the preceding pages, numerous basic substances have been isolated from the urine of patients suffering from different febrile diseases; but these, being foreign to the subject of this book, need only be alluded to here.

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